DECEMBER 10, 1942

THE JOURNAL OF NUTRITION

VOLUME 24 NUMBER 6

GEORGE R. COWGILL, *Editor*
Yale University School of Medicine, New Haven, Conn.

HAROLD H. MITCHELL, *Associate Editor*
University of Illinois, Urbana, Ill.

EDITORIAL BOARD

ICIE MACY-HOOBLER HOWARD B. LEWIS ROLAND M. BETHKE
HENRY A. MATTILL ALFRED T. SHOHL FRED A. HITCHCOCK
LELA E. BOOHER GEORGE O. BURR E. M. NELSON
CONRAD A. ELVEHJEM RUSSELL M. WILDER

Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA.
Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

Entered as second-class matter January 20, 1934, at the post office at Philadelphia, Pa., under Act of March 3, 1879. Copyright 1942, The Wistar Institute of Anatomy and Biology. All rights reserved.

Has YOUR

Subscription Expired?

This is the last number of

THE JOURNAL OF NUTRITION

to be published in 1942, and with it most subscriptions expire.

If you have not already renewed, turn to page 535, tear out order card, sign and mail — no postage required.

NUTRITION OF THE GUINEA PIG [1]

H. A. SOBER, G. J. MANNERING, M. D. CANNON, C. A. ELVEHJEM AND E. B. HART

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

(Received for publication July 10, 1942)

Although the guinea pig has long been used in studies on vitamin C, little has been done on the other nutritional requirements of this animal. These requirements have been studied in our laboratory since 1938, when the guinea pig was adopted for assay of the grass juice factor (Kohler, Elvehjem and Hart, '38). In an endeavor to replace the mineralized winter milk used as the basal diet in this assay, recourse was made to simplified dry rations which could be reproduced at any time and place, and which would give greater latitude to the investigation (Kohler, Randle, Elvehjem and Hart ('39).

Three groups of authors (Goettsch and Pappenheimer, '31; Madsen, McCay and Maynard, '33, '35; Hogan and Ritchie, '34) had previously reported experiments with guinea pigs fed synthetic rations of the casein-dextrin type containing varying amounts of yeast and supplemented with sources of vitamins A, C, D, and E. All these rations supported good growth in rats. However, the first two groups of investigators obtained poor or no growth with guinea pigs,

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by grants from Cerophyl Laboratories, and the Research Funds of the University.

We are indebted to Merck and Company, Rahway, New Jersey, for the synthetic vitamins; to Abbott Laboratories, North Chicago, Illinois, for haliver oil; to Wilson Laboratories, Chicago, Illinois, for liver extracts, and to the Works Progress Administration for assistance in the care of the animals.

THE JOURNAL OF NUTRITION, VOL. 24, NO. 6
DECEMBER, 1942
Copyright 1942
The Wistar Institute of Anatomy and Biology
All rights reserved

the animals usually dying in a relatively short period. Hogan and Ritchie also found poor growth and survival on the same type of ration with guinea pigs but obtained good growth for considerable periods when the amount of yeast was increased to 15% (as against 5% to 8% used by the first two groups of authors) plus 2% of tikitiki.

Kohler et al. ('39) reported a ration, P-5 (table 1), likewise containing dextrin and yeast, which alone would not support

TABLE 1

Rations used.

CONSTITUENTS	P-5	S-25	S-27	S-28
Dextrin, grams	59			
Sucrose, grams		74	74	62
Casein (reprecipitated 4 times), grams	18	18	18	30
Liver extract powder 1–20, grams		2		
Yeast (Pabst brewer's), grams	6			
Salts IV (Hegsted et al., '41), grams	4	4	4	4
Lard, grams	12			
Corn oil, grams		2	4	4
Cod liver oil, grams	1			
Thiamine, micrograms		200	250	250
Pyridoxine, micrograms		200	250	250
Riboflavin, micrograms			350	350
Pantothenic acid, micrograms			1000	1000
Nicotinic acid, milligrams			5	5
Choline, milligrams			400	400
Ascorbic acid	10 mg. per day		Fed by pipette	
α-Tocopherol acetate	1 mg. per week			
Haliver oil	4 drops per week			

guinea pigs, but which gave good growth after the addition of 20% of dried grass. They noted in several instances, however, that animals receiving this ration plus grass suddenly sickened during rapid growth and died. It was found that these animals had stomach ulcers. The authors postulated that this ration was deficient in at least two factors, namely, the grass juice factor and a factor necessary for the maintenance of the stomach lining.

The results to be reported in this paper have been obtained over a period of 3 years through the use of over 750 guinea

pigs. During this time the approach has changed from the development of a ration for grass juice assay purposes to a study of the nutritional requirements of the guinea pig.

EXPERIMENTAL

Guinea pigs were purchased from two local dealers during the first 2 years of this investigation. It finally became necessary to obtain guinea pigs from a large commercial breeder. All pigs were received at weights ranging from 200–300 gm. and were started immediately on experiment. They were distributed equitably as regards weight and sex among the groups comprising a given run. In most of the work five animals were employed per group.

The guinea pigs were kept in a humidity-controlled, air-conditioned room at 80° ± 2°F. The pigs were housed in metal cages, 15 inches by 20 inches (five pigs) or 10 inches by 15 inches (one pig), with ½ inch mesh screen bottoms. The animals were weighed three times weekly and fed and watered daily.

Water and ration were fed in 1-pound butter jars during the first 2½ years. Recently water bottles and metal hoppers for the ration have been substituted in an effort to reduce coprophagy. The composition of the rations is shown in table 1.

When the basal ration was supplemented with appreciable amounts of dry material, the supplements were mixed into the ration at the designated level and the sucrose content was correspondingly decreased. The yeast [2] and dried grass [3] were stored at −7°C. until used and were checked from time to time for grass juice factor activity by the mineralized winter milk assay (Kohler et al., '38; Randle et al., '40). The rations were mixed weekly and stored in the refrigerator.

Supplements amounting to less than 2 ml. per day were fed by pipette. Halibut liver oil, ascorbic acid, α-tocopherol

[2] The yeast used to supplement the sucrose rations was in all cases Northwestern Yeast Foam.

[3] From the Cerophyl Laboratories.

acetate and concentrated milk fractions were fed in this manner. When the amount of milk or grass juice was too large to be fed by pipette, the supplement was introduced into the cage in a small dish, and the pigs deprived of water until the supplement was consumed. At the conclusion of the experimental period all the surviving animals were sacrificed and autopsied.

RESULTS

Much of the preliminary work is not included and only the more significant studies are reported in table 2. These results are expressed for a 7-week test period, though some animals have been continued longer on the experiment. The great majority of the experiments have been repeated several times and the approximate number of trials may be derived from table 2 by dividing the number of animals by 5. Whenever possible, groups and rations which were essentially equivalent have been combined for conciseness. The total growth has varied from run to run, but the growth differences within a single run, due to a given supplement, were always clearly discernible.

Dextrin rations P-5

The addition of 10% of grass (or equivalent amounts of grass juice) to ration P-5 (table 1) produced an appreciable growth response which, however, was not as great as that described by Kohler et al. ('39) who used this ration supplemented with 20% of dried grass. The feeding of 20 ml. of winter milk, low in the grass juice factor, with ration P-5 + 10% grass, not only tended to decrease the incidence of stomach ulcers, but also increased the rate of growth. This would suggest that P-5 was lacking not only in the factor supplied by grass but also in a factor supplied by milk. However, since the addition of winter milk alone to ration P-5 produced considerable growth, it was concluded that this ration must have contained some source of the grass juice factor. Ration P-5 was therefore discontinued and work was initiated with rations of a more synthetic nature.

TABLE 2

Growth of guinea pigs on synthetic rations.

RATION PLUS SUPPLEMENTS	ANIMALS PER GROUP [1]	SURVIVORS AT 7-WEEKS	AVERAGE SURVIVAL [1]	AVERAGE GROWTH [2]
				gm./day
P-5	19	8	81	0.1
P-5 + 20 ml. milk	45	35	88	1.8
P-5 + 10% grass or ≏ amount of grass juice	18	12	85	2.0
P-5 + 10% grass + 20 ml. milk	12	7	86	3.2
S-25	30	14	81	—0.8
S-25 + 20 ml. milk	30	24	92	—0.1
S-25 + 10% grass	15	11	86	1.2
S-25 + fresh grass — 20 gm./day	6	6	100	1.9
S-25 + grass juice ≏ 20 gm./day fresh grass	23	9	68	—0.2
S-27	8	0	52	
S-27 + 20 ml. milk	9	4	80	0.4
S-27 + 8% yeast	8	3	74	0.6
S-27 + 8% yeast + 20 ml. milk	9	6	86	1.5
S-27 + 8% grass	8	3	67	0.7
S-27 + 8% yeast + 8% grass	9	3	72	1.5
S-27 + 8% yeast + 8% grass + 20 ml. milk	9	8	96	3.0
S-27 + 4% yeast + 4% grass + 20 ml. milk	10	7	97	1.6
S-28	20	1	57	
S-28 + 20 ml. milk	14	9	85	0.8
S-28 + 8% yeast	6	4	84	1.1
S-28 + 8% yeast + 20 ml. milk	10	5	89	0.9
S-28 + 16% yeast	4	3	72	0.7
S-28 + 16% yeast + 20 ml. milk	5	5	100	2.3
S-28 + 8% grass	10	1	60	0.8
S-28 + 8% grass + 20 ml. milk	15	8	87	2.1
S-28 + 16% grass	10	7	83	2.0
S-28 + 16% grass + 20 ml. milk	15	9	84	2.1
S-28 + 4% yeast + 4% grass	63	24	75	1.0
S-28 + 4% yeast + 4% grass + 20 ml. milk	72	49	86	1.8
S-28 + 8% yeast + 8% grass	24	16	89	2.1
S-28 + 8% yeast + 8% grass + 20 ml. milk	19	18	98	3.1
S-28 + 16% yeast + 16% grass	4	3	97	2.2
S-28 + 16% yeast + 16% grass + 20 ml. milk	5	5	100	3.8
S-28 + 10% whole dried kidney	5	0	55	
S-28 + 10% kidney + 8% grass	9	3	83	0.7
S-28 + 10% kidney + 8% grass + 20 ml. milk	9	3	78	1.5

[1] Only those animals are included which survived the first 2 weeks. Average survival = $\frac{\text{weeks survived}}{7 \text{ weeks}} \times 100$.

[2] Calculated by subtracting the second week's average weight from the seventh week's average weight and dividing by 42. Only those animals which survived 7 weeks are included in the average growth column.

Sucrose rations

These rations were patterned after the highly purified diets widely used in rat experiments. S-25 (table 1), a ration producing good growth when fed to rats (Black, Frost and Elvehjem, '40; Unna, Richards and Sampson, '41), would not support guinea pigs for any length of time. The addition of either milk or grass to S-25 increased the survival of the guinea pigs, but in the case of the milk addition there was no significant increase in growth. Grass induced some growth but the response obtained was moderate. Since only 2% of liver extract powder was included in S-25, the B complex content of the ration might have been the limiting factor. However, raising the liver extract to 4% increased the survival only slightly and did not appear to have much effect on growth. Other workers in this laboratory (Henderson, McIntire, Waisman and Elvehjem, '42) had found that they were able to raise albino rats to maturity and to obtain several generations on a synthetic ration in which the vitamin requirements were supplied by pure crystalline compounds and halibut liver oil. This ration, S-27 (table 1), was used in further investigations into the nutritional requirements of the guinea pig. Ration S-27 was unable to support growth in guinea pigs, all dying within 4 weeks. The addition to this basal ration of either 8% of dried grass, 8% of yeast or 20 ml. of winter milk resulted in slight growth responses and definitely prolonged survival. A combination of 8% of yeast and 8% of grass resulted in an additive growth response. However, when 20 ml. of winter milk were added to the basal ration S-27 + 8% of yeast + 8% of grass, even better growth resulted. The various combinations of the three supplements indicated that only in the presence of all three was the best growth obtained (table 2).

In order to preclude the stimulatory effect of milk being due to its protein or amino acid content, the amount of casein in the ration was raised from 18% to 30% (ration S-28, table 1). This ration did not permit guinea pigs to survive

for any longer period than did S-27, nor did the increased protein level remove the need for either yeast, grass, or milk. However, the hair coat and general condition of the animals appeared to be better and therefore S-28 was substituted as the basal ration in further studies. Since it has been reported that cystine may be a limiting factor for growth of the rat when casein is the sole protein source (Mulford and Griffith, '42), 0.1% of cystine was added to various combinations of the basal ration supplemented with 4% of yeast, 4% of grass, and 20 ml. of winter milk. The addition of cystine did not exert a beneficial effect. Half of the casein in S-28 was replaced by wheat gluten to test the effect of another source of protein with a different distribution of amino acids. No beneficial effect of wheat gluten was observed when this modified ration was fed with and without yeast, grass and winter milk in all the possible combinations.

Higher levels of individual supplements were fed. Sixteen per cent of either yeast or grass with and without milk were added to the basal ration. Even at these levels none of the individual supplements supplied all of the growth essentials for the guinea pig, since the addition of milk to these high levels of yeast or grass resulted in improvement of growth and survival. In the presence of both 16% of grass and 16% of yeast the addition of 20 ml. of winter milk caused an increase in growth from 2.2 gm. to 3.8 gm. per day for a period of 7 weeks (table 2).

The chick, a more rapidly growing animal than the rat, has been shown to require some factors supplied by whole dried kidney that are not required by the dog or the rat (Hegsted, Mills, Elvehjem and Hart, '41). Supplementing S-28 with 10% dried whole kidney failed to make it adequate for normal growth or development of guinea pigs. Even in the presence of 8% of grass and 20 ml. of winter milk, the addition of whole dried kidney was unable to produce maximum growth.

Since the growth response by the addition of 20 ml. of milk to either S-28 + 4% of yeast + 4% of grass, or S-28 + 8% of yeast + 8% of grass was quite appreciable and consistent, these two rations were used as assay rations for preliminary fractionation of winter milk. It was found that the factor contained in winter milk was water soluble, since it was present in the skim milk and whey fractions of milk and not in the butterfat fractions. From evidence at hand it appears that the factor was not precipitated by four volumes of 95% ethyl alcohol, nor was it extracted from whey by continuous ether extraction for 48 hours. It was stable to autoclaving for 1 hour.

In an attempt to identify the factor present in milk, the newer growth factors suggested for various species were added either singly or in combination to the basal ration, and to the basal ration plus 4% of yeast and 4% of grass in the presence and absence of the milk supplement. The following substances fed daily had no consistent effect: 40 mg. inositol, 80 mg. asparagine, 1 mg. glutamine, 600 μg. traumatic acid, and 600 μg. para-aminobenzoic acid.

DISCUSSION

Relatively little is known of the performance of herbivora on diets of known chemical composition since the rat has always been the conventional experimental animal in nutritional research. Since this investigation was initiated, several workers have published studies on guinea pigs fed sugar-casein rations. Similar to the results obtained here, they found that rations so supplemented as to produce good growth in the rat were inadequate for growth and maintenance in the guinea pig. Cannon and Emerson ('39) and Clark ('41) utilized 10% of yeast or yeast extract to supply the B complex. Both reported, however, the necessity of adding grass, but neither mentioned the need of factors other than those found in grass. Cannon and Emerson used a short experimental period and were concerned only with the immediate responses to crude foods or fractions fed. Clark used much larger animals, and his ration (no. 4) also differed in that it

contained soybean oil. Woolley ('42) has reported evidence indicating that guinea pigs require at least three dietary essentials besides those now available in pure form, namely, dried grass, a 50% ethyl alcohol soluble and a 50% ethyl alcohol insoluble fraction of linseed oil meal.

The growth reported by Clark ('41) on ration 5 and by Kohler et al. ('39) may be explained by the high level of dried grass included in their rations, since guinea pigs can grow well and reproduce when whole dried grass is their only food (Kohler, private communication). However, probably equally important is the presence of dextrin in their rations. It has long been known that rats feeding on dextrin can obtain more of the B-complex through coprophagy than those feeding on sucrose (Guerrant, Dutcher and Tomey, '35). Cannon and Emerson ('39) reported preliminary experiments indicating that guinea pigs on dextrin-containing rations would grow for considerable periods without grass. Since coprophagy is frequent in guinea pigs, it seems quite possible that, in the presence of dextrin, this species may obtain much of their special requirements from an enteric source.

The evidence presented here indicates that our guinea pigs fed a sucrose, casein, salt mixture, corn oil ration, supplemented with halibut liver oil and the known crystalline vitamins, require additional factors present in yeast, grass, and milk. The interrelationship of the three factors is illustrated in table 2. It can be seen that the single addition of milk, grass, or yeast to the basal ration, although increasing considerably the survival time, had little effect on growth. Combination of any two of these substances resulted in somewhat improved growth, but only after all three were simultaneously added to the ration, was an approximation to normal growth obtained.

With the exception of vitamins A, C, and E, very little is known of the quantitative requirements of the guinea pig for the crystalline vitamins. These were fed at a level above that known to be sufficient for the rat. Materials which are rich sources of the B complex, e.g., liver and kidney, were

added in amounts that would double, if not triple, the total vitamin content of the ration. The addition of these substances to the basal ration did not nullify the effect of the subsequent addition of yeast, milk, or grass.

The nutrient factors contributed by yeast, milk or grass are probably not identical with the supplementary factors required by the rat and may not be identical with the additional factors required by the chick. Four per cent of liver extract powder added to similar synthetic rations will produce maximal growth in rats (Black et al., '40; Unna et al., '41). A supplement of 10% of dried kidney to the same ration will supply all of the chick requirements (Hegsted et al., '40). Neither of these materials could replace yeast, grass, or milk, as supplements promoting the growth of guinea pigs on S-28.

Kohler et al. ('39), Cannon and Emerson ('39) and Clark ('41) all postulated that the factor in grass required by guinea pigs on dry rations was the same as that described by Kohler et al. ('36, '37, '38) and Randle et al. ('40), namely, the grass juice factor. It is our belief that this is true. However, in addition to the grass juice factor, "folic acid" [4] abundant in fresh grass and grass juice may also be involved, as may biotin.

Some precedent for a special factor in yeast exists in the literature. Hogan and Ritchie ('34), Kohler and co-workers ('39), Cannon and Emerson ('39) and Clark ('41) included yeast or yeast extract in their rations. As yet, neither biotin nor "folic acid" has been excluded as the factor or factors present in yeast. Now that crystalline biotin has been made available its possible application to guinea pig nutrition may be investigated.

The results reported in this paper indicate that the factor supplied by milk is not a protein constituent. Yeast has been fed to guinea pigs at a level of 16% and since yeast is approximately 40% protein, the total protein content of the ration was approximately 37%. It seems highly improbable that the guinea pig would require amounts of amino acid that were not supplied by a mixture of 30% casein and 16% dried yeast.

[4] The "folic acid" assays were kindly performed by Dr. Brian L. Hutchings.

Although rigorous proof of the non-identity of biotin or "folic acid" with the factor contained in 20 ml. of winter milk has as yet not been obtained in this laboratory, presumptive evidence exists. Winter milk has been assayed by the methods of Hutchings, Bohonos and Peterson ('41) and found to be very low in folic acid activity. The casein that is used in the ration (reprecipitated four times) is relatively unpurified. It might be expected that if the milk contained large amounts of biotin, the casein would carry sufficient biotin for the needs of the guinea pig.

SUMMARY

Evidence is presented for the existence of three factors required by the guinea pig in addition to the factors that have been demonstrated to be required by the rat and the chick; namely:

1. A factor or factors supplied by 16% of grass which is not supplied in adequate amounts by 16% of yeast and 20 ml. of winter milk.
2. Some factor or factors supplied by 16% of yeast which is not supplied in adequate amounts by 16% of grass and 20 ml. of winter milk.
3. Some factor or factors supplied by 20 ml. of winter milk which is not supplied in adequate amounts by 16% of grass and 16% of yeast.

After the preparation of this paper an article by Hogan and Hamilton appeared (Hogan, A. G., and Hamilton, J. W., J. Nutrition, vol. 23, p. 533, 1942). These authors successfully reared guinea pigs on simplified rations which contained dried yeast as the source of all the water-soluble vitamins. They are of the impression that, because of their success with yeast, the non-existence of what we have called the "grass juice factor" is probable. It should be pointed out that we have unpublished data on the distribution of the "grass juice factor" showing that it is contained in yeasts as measured by the winter milk assay, and that the particular yeast we used [5] in this study is a fair source of this factor.

[5] See footnote 2, page 505.

LITERATURE CITED

BLACK, S., D. V. FROST AND C. A. ELVEHJEM 1940 The relationship of vitamin B_6 and pantothenic acid to factor W studies. J. Biol. Chem., vol. 132, p. 65.

CANNON, M. D., AND G. A. EMERSON 1939 Dietary requirements of the guinea pig with reference to the need for a special factor. J. Nutrition, vol. 18, p. 155.

CLARK, W. G. 1941 Maintenance of adrenalectomized guinea pigs. Proc. Soc. Exp. Biol. and Med., vol. 46, p. 253.

GOETTSCH, M., AND A. M. PAPPENHEIMER 1931 Nutritional muscular dystrophy in the guinea pig and rabbit. J. Exp. Med., vol. 54, p. 145.

GUERRANT, N. B., R. A. DUTCHER AND L. F. TOMEY 1935 The effect of the type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. J. Biol. Chem., vol. 110, p. 233.

HEGSTED, D. M., J. J. OLESON, C. A. ELVEHJEM AND E. B. HART 1940 The essential nature of a new growth factor and vitamin B_6 for chicks. Poultry Science, vol. 19, p. 167.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in the nutrition of chicks. J. Biol. Chem., vol. 138, p. 459.

HENDERSON, L. M., J. M. MCINTIRE, H. A. WAISMAN AND C. A. ELVEHJEM 1942 Pantothenic acid in the nutrition of the rat. J. Nutrition, vol. 23, p. 47.

HOGAN, A. G., AND W. S. RITCHIE 1934 Nutritional requirements of rabbits and guinea pigs. Missouri Agr. Exp. Sta. Res. Bul. 219.

HUTCHINGS, B. L., N. BOHONOS AND W. H. PETERSON 1941 Purification and properties of an eluate factor required by certain lactic acid bacteria. J. Biol. Chem., vol. 141, p. 521.

KOHLER, G. O., C. A. ELVEHJEM AND E. B. HART 1936 Growth stimulating properties of grass juice. Science, vol. 83, p. 445.

———— 1937 Further studies on the growth promoting factors associated with summer milk. J. Nutrition, vol. 14, p. 131.

———— 1938 The relation of the "grass juice factor" to guinea pig nutrition. J. Nutrition, vol. 15, p. 445.

KOHLER, G. O., S. B. RANDLE, C. A. ELVEHJEM AND E. B. HART 1939 Simplified rations for guinea pigs suitable for assay of the grass juice factor. Proc. Soc. Exp. Biol. and Med., vol. 40, p. 154.

MADSEN, L. L., C. M. MCCAY AND L. A. MAYNARD 1933 Possible relationship between cod liver oil and the muscular degeneration of herbivora fed synthetic diets. Proc. Soc. Exp. Biol. and Med., vol. 30, p. 1434.

———— 1935 Synthetic diets for herbivora with reference to the toxicity of cod liver oil. N. Y. Agr. Sta. Memoir No. 178.

MULFORD, D. J., AND W. H. GRIFFITH 1942 Choline metabolism. VIII. The relationship of cystine and methionine to the requirement of choline in young rats. J. Nutrition, vol. 23, p. 91.

RANDLE, S. B., H. A. SOBER AND G. O. KOHLER 1940 The distribution of the "grass juice factor" in plant and animal materials. J. Nutrition, vol. 20, p. 459.

UNNA, K., G. V. RICHARDS AND W. L. SAMPSON 1941 Studies on nutritional achromotrichia of rats. J. Nutrition, vol. 22, p. 553.

WOOLLEY, D. W. 1942 Some new dietary essentials required by guinea pigs. Federation proc., Am. Soc. of Exp. Biol., vol. 1, no. 1, part II, p. 193.

PYRIDOXINE DEFICIENCY IN CHICKS

SAMUEL LEPKOVSKY AND F. H. KRATZER
Division of Poultry Husbandry, College of Agriculture, University of California, Berkeley

ONE FIGURE

(Received for publication July 6, 1942)

Pyridoxine has been shown to be essential for the growth of chicks by several investigators (Carter and O'Brien, '39; Jukes, '39; Hegsted et al., '39, '40; Hogan et al., '41). Jukes ('39) also reported that pyridoxine-deficient chicks showed nervous symptoms, consisting of various convulsive movements. There have been no reports concerning the occurrence of fits and convulsions in chicks such as have been described for rats (Chick, El Sadr and Worden, '40; Lepkovsky, Krause and Dimick, '42; Daniel, Kline and Tolle, '42), dogs (Fouts, Helmer, Lepkovsky and Jukes, '38) and pigs (Chick, Macrae, Martin and Martin, '38). This may have been due to the use of experimental diets deficient in factors other than pyridoxine. We have, therefore, attempted to develop a pyridoxine-deficient diet for chicks which, in so far as is possible, is deficient only in pyridoxine so that uncomplicated pyridoxine deficiency may be studied.

The difficulty of preparing a pyridoxine-deficient diet for chicks involved the inclusion in the basal diet of all necessary factors other than pyridoxine. To do this, wheat bran and yeast, treated to remove pyridoxine, were included in the basal diet. The removal of the pyridoxine also removed other factors; among them were thiamine, riboflavin and choline. These three vitamins were added to the basal ration in synthetic form. By this treatment it was hoped to include in the basal diet a maximum number of essential factors for the chick other than pyridoxine.

EXPERIMENTAL

Care of the chicks. Day-old White Leghorn chicks were fed a standard chick mash for 8 days before they were separated with respect to weight and started on the experimental diets. They were housed in electrically heated, wire-floored batteries and were given feed and water ad libitum.

Treatment of yeast [1] *and wheat bran.* The wheat bran was allowed to autolyze in water. Toluol was used as a preservative. The autolysis was carried out for a month at room temperature, after which the bran was extracted three times with water. A wine press was used to press out the water from the bran. Bakers' yeast was similarly autolyzed with water and toluol at room temperature for 3 weeks. The yeast was then extracted twice with methyl alcohol and the methyl alcohol removed from the extract by distillation at reduced pressure. The extracts of the wheat bran and yeast were separately acidified to pH 4.0 with concentrated sulfuric acid and treated five times with fuller's earth, the amount of fuller's earth used for each treatment being equivalent to half of the amount of estimated solids present in the extract. The fuller's earth was removed by filtration and the treated extracts were then concentrated at reduced pressure. The extracted yeast and wheat bran were dried and added to the diets with their fuller's earth-treated extracts in amounts equivalent to the original dry material.

Preparation of the diet. The basal diet contained the following per 100 gm.: extracted wheat bran + fuller's earth-treated wheat bran extract, 10; extracted yeast + fuller's earth-treated yeast extract, 5; water-washed casein, 15; gelatin, 5; glucose,[2] 50.45; calcium gluconate, 5; fish oil, 0.25; soybean oil, 3; cottonseed oil [3] containing 1% vitamin E distillate,[4] 2; sodium chloride mixture containing 0.49% manganese, 0.1% iron, 0.05% copper, 0.05% zinc, 0.05% aluminum,

[1] Fresh bakers' yeast was donated for this work by Standard Brands, Inc.
[2] Cerelose.
[3] Wesson.
[4] The vitamin E distillate was donated by Distillation Products, Inc.

0.002% cobalt, and 0.04% iodine, 1; tricalcium phosphate, 2; dipotassium phosphate, 0.5; potassium chloride, 0.3; magnesium sulfate, 0.1; sodium silicate, 0.2; cholic acid, 0.1; choline, 0.1; hexane extract of alfalfa equivalent to 2% alfalfa; thiamine,[5] 0.5 mg.; and riboflavin,[5] 0.5 mg.

Ten groups of pyridoxine-deficient chicks totalling eighty-one were used, and another ten groups totalling fifty-eight chicks were fed the deficient diet plus 2 mg. per kilo of pyridoxine. Though there were some variations in the pyridoxine-deficient diets used, the behavior of the chicks was sufficiently uniform so that only one of the groups need be described in detail.

RESULTS

After a small initial gain in weight (table 1) the pyridoxine-deficient chicks ceased to grow or grew very slowly. Out of the seven chicks in the illustrative groups under discussion,

TABLE 1

The effect of pyridoxine upon growth and occurrence of convulsions in chicks. Illustrative data.

DIET	NO. CHICKS	AV. WEIGHT AT START	AV. WEIGHT AFTER 3 WKS. ON DIET	NUMBER SURVIVORS	NO. CHICKS OBSERVED IN CONVULSIONS
Basal	7	64.4	83.8	5	7
Basal + 2 mg. pyridoxine per kilogram	8	65.0	179.7	8	0

two had convulsions after they were on the deficient diet for 12 days. On the fourteenth day one chick died in a convulsion. On the sixteenth day another chick died after several convulsions, the first of which occurred on the twelfth day. Convulsions in the remaining five chicks were occasionally observed until they had been on the diet for 24 days after which no convulsions were noted. The chicks were weak and, for the most part, squatted in the cages with wings slightly extended from their bodies, and their heads resting on the wire screen. The wing feathers seemed to grow faster than

[5] The thiamine and riboflavin were donated by Merck and Co.

the bodies of the chicks and sometimes extended beyond the tail feathers. There was little feather growth on the body, the down remaining for the most part. No signs of dermatitis were observed. Some of the positions taken by the chicks are shown in figure 1. At times some chicks shivered as though

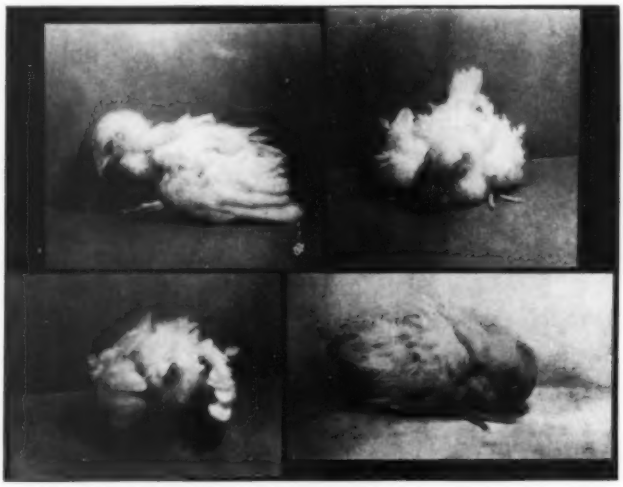


Fig. 1 Positions assumed by pyridoxine-deficient chicks.

they were cold, but the chicks themselves, as well as their surroundings were warm. The tails of some chicks vibrated very rapidly. The chicks walked with a stiff high-stepping gait.

After 32 days, one of the remaining five chicks died, and two others died after 45 days. At this time the remaining two chicks were given 1 mg. each of pyridoxine. One died and the other gained 26 gm. in 2 days, after which it went into a continuous convulsion which lasted until the chick died about 2 days later.

The control chicks fed 2 mg. pyridoxine [6] per kilo of diet grew rapidly (table 1) and appeared normal for about 17

[6] The pyridoxine was donated by Merck and Co.

days, after which the rate of growth decreased in some of them. They were discarded after they were on the diet for 21 days. The control chicks were not quite normal, indicating the existence of a deficiency of a factor or factors other than pyridoxine.

Pyridoxine-deficient syndrome. The behavior of the pyridoxine-deficient chicks could well be divided into three different stages. In stage 1, all that could be noticed in the chicks was an abnormal excitability. Stage 2 was characterized by jerky, convulsive movements. Chicks would suddenly run aimlessly, often flapping their wings and keeping their heads down. Since the chicks were weak, these sudden, quick dashes were all the more surprising. In these dashes the chicks did not appear to be conscious of direction, since they often dashed off the end of a table. The eyes were apparently normal.

In stage 3 convulsions occur. While all the convulsions are not similar, they are nevertheless characteristic. Sometimes while resting on their breasts the chicks will raise their feet off the floor and flap their wings. Sometimes they will rapidly tap the floor with their feet while squatting. At times they will hop, largely with the assistance of their wings. During a convulsion they may fall on their sides or roll over on their backs and rapidly paddle with their feet. Their heads often jerk up and retract as in polyneuritis, sometimes move convulsively in an up-and-down movement, with the neck distended or twisted. The mandibles sometimes open and close rapidly. In severe convulsions, the chick enters a state of complete exhaustion which at times resembles a comatose state. After a period of time, varying from a few seconds to about 2 minutes, the chick usually recovers and becomes apparently normal. Convulsions will occur when the chicks are in the cages without any obvious disturbance. After their peculiar dashes, chicks will sometimes go into severe convulsions. In general, there seems no way of forecasting a convulsion, nor is there any certain method of bringing it about.

DISCUSSION

The production of convulsions in pyridoxine-deficient chicks would seem to endow pyridoxine with a uniformity of function in different animals, similar to that of thiamine. It seems that before distinct convulsions occur, the basal ration must be relatively free of deficiencies other than that of pyridoxine. The complete absence of dermatitis is significant and is also in harmony with the decreased emphasis on dermatitis as a characteristic feature of pyridoxine-deficiency in rats (Lepkovsky and Krause, '42). The weakness of the deficient chicks and the shivering of some of them would seem to resemble the clinical findings of weakness (Spies, Bean and Ashe, '39) and paralysis agitans (Jolliffe, '41) in the human.

The promptness with which convulsions occur in chicks, after about 12 days on the deficient diet, is in striking contrast to the 4 to 6 months that is required by rats to develop similar convulsions.

SUMMARY

1. The preparation for chicks of a diet deficient chiefly in pyridoxine has been described.

2. Pyridoxine deficiency in chicks is characterized by slow growth, convulsions, and other nervous manifestations.

3. No dermatitis of any kind was observed in the pyridoxine-deficient chicks.

LITERATURE CITED

CARTER, C. W., AND J. R. O'BRIEN 1939 Vitamin B complex in relation to the nutrition of the chick and pigeon. Proc. Seventh World's Poultry Congress and Exposition, Cleveland, Ohio, p. 126.

CHICK, H., T. F. MACRAE, A. J. P. MARTIN AND C. J. MARTIN 1938 The water soluble B vitamins other than aneurin (vitamin B_1), riboflavin and nicotinic acid required by the pig. Biochem. J., vol. 32, p. 2207.

CHICK, H., M. M. EL SADR AND A. N. WORDEN 1940 Occurrence of fits of an epileptiform nature in rats maintained for long periods on a diet deprived of vitamin B_6. Biochem. J., vol. 34, p. 595.

DANIEL, E. P., O. L. KLINE AND C. D. TOLLE 1942 A convulsive syndrome in young rats associated with pyridoxine deficiency. J. Nutrition, vol. 23, p. 205.

FOUTS, P. J., O. M. HELMER, S. LEPKOVSKY AND T. H. JUKES 1938 Production of microcytic hypochromic anemia in puppies on synthetic diet deficient in rat antidermatitis factor (vitamin B_6). J. Nutrition, vol. 16, p. 197.

HEGSTED, D. M., J. J. OLESON, C. A. ELVEHJEM AND E. B. HART 1939 The "cartilage growth factor" and vitamin B_6 in the nutrition of chicks. J. Biol. Chem., vol. 130, p. 423.

———— 1940 The essential nature of a new growth factor and vitamin B_6 for chicks. Poul. Sci., vol. 19, p. 167.

HOGAN, A. G., L. R. RICHARDSON, H. PATRICK, B. L. O'DELL AND H. L. KEMPSTER 1941 Vitamin B_6 and chick nutrition. Poul. Sci., vol. 20, p. 180.

JOLLIFFE, N. 1941 Newer knowledge of the vitamin B complex. Bull. New York Acad. Med., vol. 17, p. 195.

JUKES, T. H. 1939 Vitamin B_6 deficiency in chicks. Proc. Soc. Exp. Biol. and Med., vol. 42, p. 180.

LEPKOVSKY, S., M. E. KRAUSE AND M. K. DIMICK 1942 The occurrence of fits in pyridoxine deficient rats. Science, vol. 95, p. 331.

LEPKOVSKY, S., AND M. E. KRAUSE 1942 Dermatitis in pyridoxine-deficient rats. Proc. Soc. Exp. Biol. and Med., vol. 49, p. 57.

SPIES, T. D., W. B. BEAN AND W. F. ASHE 1939 A note on the use of vitamin B_6 in human nutrition. J. Am. Med. Assoc., vol. 112, p. 2414.

RESPONSE OF BACTERIA, YEAST AND RATS TO PEROXIDE-TREATED BIOTIN. INTESTINAL SYNTHESIS OF BIOTIN IN THE RAT[1]

EDWARD NIELSEN, G. M. SHULL, AND W. H. PETERSON
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

ONE FIGURE

(Received for publication July 6, 1942)

Biotin has recently acquired an important position in animal nutrition because of its connection with so-called "egg white injury." Egg white injury was first encountered by Boas ('27) and studied extensively by Parsons ('31), Parsons and Kelly ('33), György ('39) and Birch and György ('39). These researches culminated in the brilliant discovery by du Vigneaud, Melville, György and Rose ('40) that biotin is the agent which counteracts the injurious effects of raw egg white. The egg white syndrome in rats resembles "pink disease" in man (Findlay and Stern, '29). Recently Sydenstricker, Singal, Briggs, De Vaughn and Isbell ('42) have reported an experimental biotin deficiency in humans, characterized by a scaly dermatitis, muscle pains and a change in the blood picture. Another aspect of nutrition involving biotin is the "spectacle eye" syndrome of the rat reported by Nielsen and Elvehjem ('41), a condition which has been studied extensively by several workers in relation to other vitamins (Oleson, Elvehjem and Hart, '40; Pavcek and Baum, '41). Biotin plays a role in fat synthesis and metabolism (Gavin and McHenry,

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the National Live Stock and Meat Board made through the National Research Council.

'41), and it has been noted that the biotin content of tumor tissue is higher than that of normal tissue (West and Woglam, '41). Studies of a dermatitis in chicks distinct from that produced by pantothenic acid deficiency have shown biotin to be involved in chick nutrition (Hegsted, Mills, Briggs, Elvehjem and Hart, '42). It is also important in the nutrition of the turkey (Patrick, Boucher, Dutcher and Knandel, '41).

In the nutrition of microorganisms biotin has held a first-rank place that antedates by several years recognition of its importance in the animal field. It was first discovered as a growth factor for yeast by Kögl and Tönnis ('36), but since that time has been found to be required by many bacteria and fungi (Robbins and Kavanagh, '41).

In developing an assay method for biotin by use of Lactobacillus casei, it was found that the constituents of the medium which had been rendered biotin-free by treatment with hydrogen peroxide showed marked biotin potency when tested by yeast. Because of this difference between the bacterial and yeast assays it seemed desirable to test the peroxide-treated biotin by animal assay. It has been found that the results with rats and with Lactobacillus casei agree in that neither organism shows any response to the peroxide-treated compound. Apparently yeast is able to regenerate biotin from the oxidation product. This is not surprising in view of the marked synthetic and reducing powers possessed by yeast. Biotin determinations on the feces and urine of the depleted rats showed such large amounts of biotin to be excreted that the work was extended to a study of the synthesis of biotin in the intestinal tract under various dietary conditions.

EXPERIMENTAL

A biotin concentrate (no. 5000)[2] was used as a source of biotin for the comparative studies. A method of peroxide-treatment described by Shull, Hutchings and Peterson ('42) was used to produce an oxidation product of biotin. The biotin treated in this manner will be referred to hereafter as

[2] Supplied by S. M. A. Corp.

"peroxide-treated biotin." The peroxide treatment used in these experiments is milder than that employed by Brown and du Vigneaud ('41). In order to ascertain the effectiveness of this treatment, an aliquot was assayed both by the yeast method [3] of Snell, Eakin and Williams ('40) and by the Lactobacillus casei method. The data are given in table 1. From these results it appears that the use of oxidizing agents must be avoided in preparing biological materials for assay by the yeast method. Whether yeast responds to other decomposition products of biotin is a question that needs further study.

TABLE 1

Response of Lactobacillus casei and yeast to peroxide-treated biotin. (Biotin in mμg./cc.)

FORM OF BIOTIN	LACTOBACILLUS CASEI			YEAST		
	Untreated	Treated	% Activity	Untreated	Treated	% Activity
Free acid	0.3	0.0	0	0.3	0.3	100
Free acid	4.0	0.0	0	4.0	3.46	89
Methyl ester	125	0.0	0	125	102	82
Concentrate (S.M.A.)	3540	2.3	< 1.0	4000	2220	55

The basal ration and the care of the animals have been described in a previous paper (Nielsen and Elvehjem, '41). In a preliminary experiment rats were placed on the basal ration modified by replacing 10% of casein with an equivalent amount of commercial egg white. After 5 weeks the egg white in the ration was reduced to 5% and kept at this level until the end of the experiment. The growth response resulting from the administration of biotin concentrates was considered to be the most accurate means to measure biotin activity. The growth curve for a typical animal appears in figure 1 as curve 1.

The growth noted over the feeding period is as follows: at the end of the fifth week the weight curve of the animal had begun to exhibit a plateau; then 1 μg. of "peroxide-treated

[3] We wish to thank Miss M. A. McGregor and Mr. J. O. Lampen for these assays.

biotin'' was administered intraperitoneally, daily, for a period of 14 days, the rat losing 5 gm. during this time. This level of ''peroxide-treated biotin'' was then fed orally for 7 days and the animal's weight did not change. During the next 7 days the rat did not receive any form of biotin. Then 1 μg. of untreated biotin was fed orally for 7 days and over the week the rat showed a gain of 48 gm.

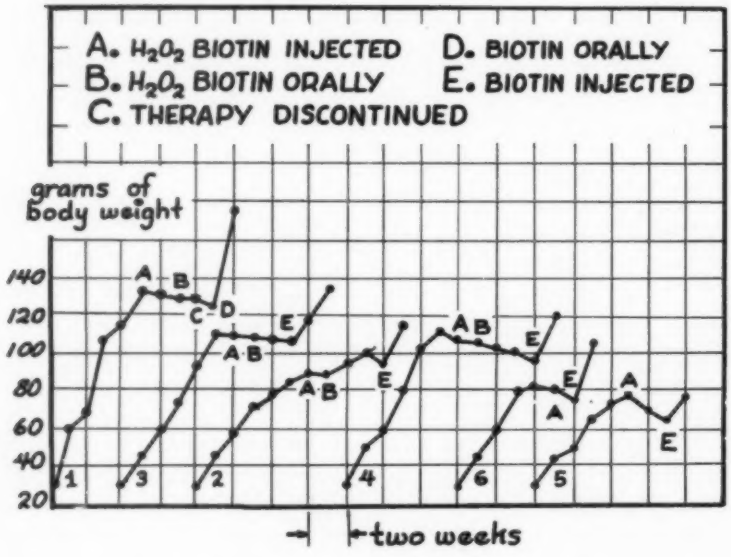


Fig. 1 Response of rats to various forms of biotin.

The ration for the five animals in the next experiment was the same as that described above, except that the commercial egg white was kept at a 10% level throughout the assay period. This level of egg white was adopted because a 5% level failed to tie up all the biotin synthesized in the intestinal tract. The first three rats were depleted of their stores of biotin over a 6-week period, then 1 μg. of ''peroxide-treated biotin'' was injected intraperitoneally for 7 days. Following this period ''peroxide-treated biotin'' was fed orally at the same level

for either 2 or 3 weeks, with the net result that all the animals were still losing or only maintaining weight. Then 1 μg. of biotin was injected daily for 7 days and marked gains in weight were obtained, as shown in figure 1, curves 2, 3 and 4.

The two additional rats were depleted for a 5-week period and then "peroxide-treated biotin" at a 1 μg. level per day was injected for 1 and 2 weeks, respectively. These two rats exhibited loss in weight, as previously observed. Then 1 μg. of biotin per day was injected and the usual responses were noted, as shown in curves 6 and 5.

From a study of the growth curves it appears that the rat is similar to Lactobacillus casei in that it cannot use this oxidized form of biotin either orally or parenterally.

The effect of oxidizing biotin, observed in this paper, is probably related to some compound less oxidized than the sulfone derivative of Hofmann, Melville and du Vigneaud ('41), since their compound does not promote the growth of yeast.

In order to study the synthesis of biotin in the intestinal tract of the rat a biotin-low ration was purified by the following procedure.

Biotin-low ration

Treatment of the components. One thousand grams of acid-washed casein were suspended in 10 gallons of water and the pH adjusted to 8.5 with NaOH. To this viscous solution 430 ml. of Superoxol were added and the solution thoroughly stirred and allowed to stand at room temperature for 24 hours; then 230 gm. of powdered MnO_2 were added slowly and the solution stirred vigorously until no more oxygen was evolved. The solution was filtered by means of a filter press and the casein precipitated with acid. The casein was freed of excess acid by washing with warm water, collected on a cloth filter bed and finally dried and powdered in the usual manner.

The sugar was purified by extracting with 100% ethyl alcohol. One kilogram of sugar was placed in a cloth bag and

inserted in a Soxhlet extractor. Sufficient 100% ethyl alcohol was added to allow a continuous extraction for 30 hours.

The percentage composition of the ration and the level of B vitamins fed are given in a previous paper (Nielsen and Elvehjem, '41). The individual components of the ration were assayed by the Lactobacillus casei method of Shull, Hutchings and Peterson ('42). The purified ration contained 63.1% less biotin than the unpurified ration, and on a 10 gm. food intake per day would supply only 0.003 μg. of biotin.

Two rats were maintained on this purified ration for 12 weeks and failed to show any symptoms of a biotin deficiency. For comparison purposes a typical rat which received commercial egg white equivalent to 10% of the ration weighed 85 gm. at the end of the seventh week; the fur was unkempt and the back arched; a littermate on the purified ration weighed 181 gm. at the end of the seventh week and appeared normal in all respects. Since the purified ration was furnishing only 0.003 μg. of biotin per day, it appeared that the rats were meeting their requirement by synthesizing this compound.

Synthesis of biotin. To obtain some data on the question of synthesis, biotin excretion studies were conducted over a 3-day period during which the rats were placed in metabolism cages in order to collect all excreta. The intake of biotin and all excretion values as determined by the Lactobacillus casei method are expressed in table 2.

Rats no. 1 and no. 2 were placed on the purified ration for 6 weeks and then the excreta were collected. The average synthesis per day was 295 mμg. These same rats were continued on the purified ration for 3 weeks longer and at the end of that period the excreta were collected as before. The average synthesis per day was 358 mμg. (rats 1 A and 2 A in table 2).

In order to study the effects of sulfaguanidine on the synthesis of biotin, rats nos. 3, 4, 5, and 6 were placed on the purified ration containing 0.5% sulfaguanidine. Sulfaguanidine (sulfanilylguanidine), an antibacterial agent which is poorly absorbed from the intestine (Marshall, Bratton, White

TABLE 2

Synthesis of biotin in the intestinal tract of rats fed various rations.
(Biotin values are expressed in mμg.)

RAT NO.	WT.	RATION			BIOTIN PER GRAM OF FECES	FECES PER DAY	BIOTIN EXCRETION — 3 DAYS			BIOTIN SYNTHESIS PER DAY
		Type	Consumed	Biotin content			Feces	Urine	Total	
	gm.		*gm.*			*gm.*				
1 (N) [1]	160	B.L.[2]	35	10.5	647	0.37	712	89	801	263
2 (N)	112	B.L.	38	11.4	532	0.53	850	144	994	328
1A (N)	219	B.L.	33	9.9	919	0.37	1010	59	1069	353
2A (N)	162	B.L.	39	11.7	509	0.67	1020	82	1102	363
3 (N)	61	S.G.	11	3.3	234	0.50	234	25	259	128
4 (N)	77	S.G.	1	0.3	483	0.18	85	36	121	121
5 (N)	75	S.G.	19	5.7	105	0.23	74	28	102	32
6 (N)	69	S.G.	19	5.7	451	0.17	225	86	311	102
7 (S.E.)	120	P.A.L.	23	18.8	678	0.30	610	130	740	240
8 (N)	83	P.A.L.	16	13.1	625	0.20	375	102	477	155
9 (S.E.)	59	P.A.L.	11	9.0	1603	0.13	642	57	699	230
10 (N)	142	P.A.L.	28	23	1117	0.28	950	136	1086	354
11 (P)	55	R.L.	8	26.9	2480	0.07	489		489	154
12 (S.E. + P.)	65	R.L.	12	40.4	1280	0.20	768		768	243
13 (S.E.)	88	R.L.	19	64.0	801	0.10	240		240	59
14 (N)	163	Stock	32	2960.0	1190	2.37	8450	390	8840	1960

[1] Symptoms: N — normal; S.E. — spectacle eye; P — paralysis.

[2] Rations: B.L. — biotin low; S.G. — sulfaguanidine; P.A.L. — pantothenic acid low; R.L. — riboflavin-low.

and Litchfield, '40), was included in this ration in the hope of reducing the number of intestinal bacteria. A 0.5% level was fed, since previous work (Black, McKibbin and Elvehjem, '41) had indicated that this level was not toxic to the rat, but did reduce the number of intestinal organisms. Excreta were collected at the end of the fifth week from rat no. 3, but unfortunately the animal lived only 2 days after the collection began. The synthesis of biotin was 128 mμg. per day. Rat no. 4 was put into the metabolism cage but died after 20 hours; synthesis for this period was 121 mμg.[4] The excretion of rats nos. 5 and 6, collected for 3 days at the end of the third week, showed synthesis of 32 mμg. and 102 mμg. per day, respectively. Sulfaguanidine tested at a level of 600 μg. per tube had no effect on the assay. Rats no. 5 and no. 6 failed to respond to the injection of 1 μg. of biotin per day over a 7-day period. This finding is not surprising since we were dealing with a complicated deficiency. These animals were deficient not only in biotin but undoubtedly in vitamin K[4] as well. Possibly additional factors elaborated by bacterial synthesis were lacking.

Rats nos. 7, 8, 9 and 10 were maintained on a basal ration deficient in pantothenic acid for a period of 6–8 weeks before the feces were collected. There appeared to be a slight decrease in the total synthesis in the case of rats nos. 8 and 9 as compared with the rats on the biotin-low ration. The intake and amount synthesized are given in table 2.

Rats nos. 11, 12 and 13 were maintained on a riboflavin-low ration for 6–8 weeks. The urine was not collected, since at this time we believed that the amount of biotin found in the urine was insignificant as compared to the total synthesis of this vitamin. There appeared to be a noticeable decrease in the amount of biotin synthesized. The intake and synthesis are given in table 2.

In order to find a value for this synthesis on a natural diet a stock animal (no. 14), comparable to the animals used in the biotin-low ration, was placed in a metabolism cage. The

[4] Rats on a similar ration were found to be deficient in vitamin K as observed by S. Black of this laboratory.

intake of biotin was very high as the stock ration was fed throughout the test period. The biotin content of the urine was fairly high and the total synthesis was 1960 mμg. per day as shown in table 2. This figure is five times the value obtained on the biotin-low ration.

Three animals receiving the basal ration with 10% of commercial egg white in place of the casein were studied. The total synthesis per day was 1315, 1410 and 1379 mμg. or approximately four times the value obtained on the biotin-low ration. Judging from these values it appears that if there is a means of binding synthesized biotin in the intestinal tract, the bacteria will produce an additional amount.

Since there is synthesis of riboflavin in voided feces of chicks (Lamoreux and Schumacher, '40), the synthesis of biotin in voided feces was studied. Feces were removed from the colon and dried in an oven at 95°C. for 24 hours to destroy bacteria. A 1-day collection of feces from the same animal was allowed to stand in the animal room for 2 days and then dried as described above. The biotin content of feces from rats on different rations was as follows: the feces of a riboflavin-deficient animal as passed contained 706 mμg., and after standing at room temperature for 2 days 949 mμg., or an increase in biotin of 34.4%. The feces of a pantothenic acid-deficient animal under the same conditions contained 843 mμg. and 769 mμg., respectively, or a decrease in biotin of 8.8%. A stock colony animal's feces as passed gave a value of 1055 mμg., but on standing increased to 1635 mμg., a gain of 54.9%. The large increase in this case can probably be attributed to the favorable substrate for bacterial growth afforded by the indigestible material in the stock animal's feces. The additional synthesis of biotin in the experimental rations was not of such a magnitude as to account for the biotin content of the feces as voided.

SUMMARY

1. Lactobacillus casei and the rat cannot utilize biotin that has been oxidized by mild treatment with hydrogen peroxide (0.3% for 24 hours at room temperature). However, yeast

does utilize biotin treated in this manner. In the assay of biological materials for biotin oxidizing conditions should be avoided in the preparation of the sample.

2. Biotin syntheses varied with different rations. It was highest on the stock ration (1960 mμg. per day per rat) and very low on a riboflavin deficient ration (152). When the sulfaguanidine was added to a biotin-low ration, the lowest value (80) was reached.

3. The preparation of a biotin-low ration has been described.

4. The synthesis of biotin in voided feces has been studied.

LITERATURE CITED

BIRCH, T. W., AND P. GYÖRGY 1939 Physicochemical properties of the factor (vitamin H) curative of egg white injury. J. Biol. Chem., vol. 131, p. 761.

BLACK, S., J. M. MCKIBBIN AND C. A. ELVEHJEM 1941 Use of sulfaguanidine in nutrition experiments. Proc. Soc. Exp. Biol. and Med., vol. 47, p. 308.

BOAS, M. A. 1927 The effect of desiccation upon the nutritive properties of egg white. Biochem. J., vol. 21, p. 712.

BROWN, G. B., AND V. DU VIGNEAUD 1941 The effect of certain reagents on the activity of biotin. J. Biol. Chem., vol. 141, p. 85.

FINDLAY, C. M., AND R. O. STERN 1929 A syndrome in the rat resembling pink disease in man. Arch. Dis. Childhood, vol. 4, p. 1.

GAVIN, G., AND E. W. MCHENRY 1941 The effects of biotin upon fat synthesis and metabolism. J. Biol. Chem., vol. 141, p. 619.

GYÖRGY, P. 1939 The curative factor (vitamin H) for egg white injury, with particular reference to its presence in different foodstuffs and in yeast. J. Biol. Chem., vol. 131, p. 733.

HEGSTED, D. M., R. C. MILLS, G. M. BRIGGS, C. A. ELVEHJEM AND E. B. HART 1942 Biotin in chick nutrition. J. Nutrition, vol. 23, p. 175.

HOFMANN, K., D. B. MELVILLE AND V. DU VIGNEAUD 1941 Characterization of the functional groups of biotin. J. Biol. Chem., vol. 141, p. 207.

KÖGL, F., AND B. TÖNNIS 1936 Über das Bios-Problam Darstellung von Krystallisiertem Biotin aus Eigelb. Z. physiol. Chem., vol. 242, p. 43.

LAMOREUX, W. F., AND A. E. SCHUMACHER 1940 Is riboflavin synthesized in the feces of fowl. Poul. Sci., vol. 19, p. 418.

MARSHALL, E. K., JR., A. C. BRATTON, H. J. WHITE AND J. T. LITCHFIELD, JR. 1940 Sulfanilylguanidine: A chemotherapeutic agent for intestinal infection. Johns Hopkins Hosp., vol. 67, p. 163.

NIELSEN, E., AND C. A. ELVEHJEM 1941 Cure of spectacle eye condition in rats with biotin concentrates. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 349.

OLESON, J. J., C. A. ELVEHJEM AND E. B. HART 1940 Spectacle eye condition in rats. Proc. Soc. Exp. Biol. and Med., vol. 43, p. 161.

PARSONS, H. T. 1931 The physiological effects of diets rich in egg white. J. Biol. Chem., vol. 90, p. 351.

PARSONS, H. T., AND E. KELLY 1933 The character of the dermatitis producing factor in dietary egg white as shown by certain chemical treatments. J. Biol. Chem., vol. 100, p. 645.

PATRICK, H., R. V. BOUCHER, R. A. DUTCHER AND H. C. KNANDEL 1941 Biotin and prevention of dermatitis in turkey poults. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 456.

PAVCEK, P. L., AND H. M. BAUM 1941 Inositol and spectacled eye in rats. Science, vol. 93, p. 502.

ROBBINS, W. J., AND VIRGENE KAVANAGH 1941 Plant growth substances. Ann. Review of Biochem., vol. 10, p. 499.

SHULL, G. M., B. L. HUTCHINGS AND W. H. PETERSON 1942 A microbiological assay for biotin. J. Biol. Chem., vol. 142, p. 913.

SNELL, E. E., R. E. EAKIN AND R. J. WILLIAMS 1940 A quantitative test for biotin and observations regarding its occurrence and properties. J. Am. Chem. Soc., vol. 62, p. 175.

SYDENSTRICKER, U. P., S. A. SINGAL, A. P. BRIGGS, N. M. DE VAUGHN AND H. ISBELL 1942 Observations on the "egg white injury" in man. J. Am. Med. Assoc., vol. 118, p. 1199.

DU VIGNEAUD, V., D. B. MELVILLE, P. GYÖRGY AND C. ROSE 1940 On the identity of vitamin H with biotin. Science, vol. 92, p. 62.

WEST, P. M., AND W. H. WOGLAM 1941 The biotin content of tumors and other tissues. Science, vol. 93, p. 525.

PERIODIC ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT AS AFFECTING THE METABOLISM OF RATS ON DIETS OF DIFFERENT COMPOSITION [1]

HELEN E. ARCHER, MAX KRISS AND LEROY VORIS
Institute of Animal Nutrition, Pennsylvania State College, State College

(Received for publication June 13, 1942)

In the presence of essential accessory food factors, the preferential oxidation or anabolism of protein, carbohydrate and fat within an animal is determined largely by the relative availability of these nutrients at the moment, and by the relative activity of various endocrine secretions in the body. In the latter capacity the secretions of the anterior pituitary undoubtedly play a dominant role, the exact nature of which is, at present, little understood.

As one approach to the study of this problem the present investigation deals with the effects of 3-day pituitary treatments at 2-week intervals on the disposition of the nutrient energy of a balanced diet and of diets characterized by excess of protein, carbohydrate or fat. Also the results are of interest in comparison with the effects of extended daily treatment with A.P.E. (anterior pituitary extract) as reported in previous papers (Voris, Kriss, Marcy and Bowman, '42 a, b) from this Institute.

Since no corresponding investigation has been reported, so far as the present authors are aware, reference to pertinent literature will be made only in the discussion of the results.

[1] Authorized for publication on June 8, 1942, as paper no. 1110 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

EXPERIMENTAL

Six litter-mate pairs of male albino rats from commercial stock,[2] weighing approximately 100 gm. at the start of the program, were used as experimental subjects. They were kept in individual metabolism cages and allowed tap water ad libitum.

One rat of each pair was injected periodically with a saline anterior pituitary extract (A.P.E.), while the other, serving as the control, was treated similarly with 1% sodium chloride solution. In all other respects both rats of a pair were treated identically.

After a preliminary period of 8 to 10 days during which the limit of voluntary food consumption was determined and the rats gained about 30 gm., they were subjected during a period of 8 weeks to the following treatments in order of sequence: (1) fasting, (2) normal stock [3] diet, (3) carbohydrate-rich diet, (4) protein-rich diet, (5) fat-rich diet, (6) fasting. During the first five treatments respiratory metabolism was measured and urine was collected. In the final fasting test the respiration measurement was omitted and urinary N only was determined as a check on the initial test. Intraperitoneal injections of the A.P.E. were given in 1 ml. portions 2 days prior to, and on the day of, the respiration measurements.

In the fasting period the rats were given food on the day of the first injection; at the time of the second injection food was withdrawn. After 24 hours of fast, and immediately after the third injection, respiration tests were started and continued for 6 hours. Urine was collected for 48 hours following the respiration measurement.

In the feeding periods, each of which lasted 2 weeks, the rats were fed twice daily and trained to eat promptly. The food intake was standardized at 10 gm. daily (5 gm. twice a day). This method of feeding assured experimental uniformity and convenience. At the end of 10 days' preliminary

[2] Sunny Hill Rat Farm, Clinton, Maryland.
[3] Purina dog chow.

feeding, the first daily injection of A.P.E. was given in the morning, and urine collection was started in the afternoon and continued for 72 hours. After the third injection, and soon after the morning meal was consumed, the respiration measurement was started.

In the preparation of the diets, the basal stock diet was used as a component of the mixed diets. The carbohydrate and protein-rich diets were composed of equal portions by weight of the basal diet and dextrin [4] or beef-muscle protein.[5] The fat-rich diet was composed of the basal diet and hydrogenated vegetable oil [6] in proportions of three to two by weight. A summary of the gross composition of each diet on the dry basis is given in table 1.

TABLE 1

Gross composition of diets on dry basis.

	STOCK	CARBOHYDRATE-RICH	PROTEIN-RICH	FAT-RICH
	%	%	%	%
Carbohydrate	55.0	77.5	27.5	22.0
Protein	27.0	13.5	63.5	10.8
Fat	9.0	4.5	4.5	63.6
Ash	9.0	4.5	4.5	3.6

At the start of each experimental period fresh anterior pituitary extract was prepared from frozen anterior pituitary lobes of cattle.[7] The methods of preparation and preservation have already been described (Voris et al., '42 a). Each milliliter of extract contained 1.5 mg. N.

Respiratory metabolism measurements were made for 6 hours by the Haldane open-circuit gravimetric technic. The apparatus was similar to that described by Forbes, Kriss and Miller ('34) with modifications reported by Kriss ('38). The total O_2 and CO_2 for the 6 hours were used in computation of the R. Q.'s. The average hourly CO_2, excluding the first hour and other hours in which activity was excessive, was used for

[4] Amidex, Corn Products Refining Co., New York.

[5, 7] Wilson Laboratories, Chicago.

[6] Crisco.

computation of heat production on the basis of O_2 consumption values derived therefrom. The gaseous exchange and the heat of protein metabolism were computed on the basis that 1 gm. of urinary N is equivalent to 4.75 liters CO_2, 5.96 liters O_2 and 26.71 Calories (Kriss and Voris, '37).

Nitrogen was determined in the individual urine samples collected from each rat during the fasting and feeding periods. Carbon and nitrogen were determined in composited urine samples from the feeding periods. Periodic tests were made for acetonuria.

RESULTS

Urinary N and C

The effects of the A.P.E. on the excretion of urinary N and C are shown in table 2. The fasting results and those on the stock diet were used previously (Voris et al., '42 b) for comparison with those obtained after extended treatment with the extract.

In all periods the urinary N excretion was reduced in the rats treated with the A.P.E. The differences were most significant with the rats on the stock and the protein-rich diets. In the first fasting period two treated rats showed responses opposite to the average, and again, in the second fasting test, the same two rats did not conform to pattern. The divergence of these two rats accounts for the high standard error of the average difference in the fasting urinary N.

It is of interest that the urinary N values relative to the intakes were quite similar for the stock and the carbohydrate-rich diets and for the protein and fat-rich diets, but the values in the first two periods were notably smaller than in the last two.

In the rats treated with the A.P.E. the urinary C excretion was decreased in each feeding period excepting that of the carbohydrate-rich diet. In all feeding periods the ratio of urinary C to N was increased. The decrements in urinary C and N were related in the proportion of 0.4 : 1, indicating de-

creased secretion of urea (C/N = 0.43). The C/N values for the carbohydrate-rich diet were higher than those on other dietary treatments, indicating a decreased proportion of urea to creatinine (C/N = 1.14) and uric acid (C/N = 1.07).

The absence of ketosis was evident both from the C/N values and the consistently negative tests for acetonuria.

TABLE 2

Daily excretion of urinary nitrogen and carbon in rats as affected by limited treatment with anterior pituitary extract.

TREATMENT	NO. OF RATS	N INTAKE	URINARY N		URINARY C	C TO N RATIO
			Amount	Per cent of intake		
		mg.	*mg.* [1]		*mg.*	
Fasting						
Treated	6		118 ± 16			
Control	6		147 ± 18			
Difference			29 ± 24			
Stock diet						
Treated	6	432	127 ± 3	29.4	113	0.89
Control	6	432	166 ± 5	38.4	130	0.78
Difference			39 ± 5	9.0	17	0.44
Carbohydrate-rich						
Treated	5	216	70 ± 3	32.4	73	1.04
Control	5	216	78 ± 3	36.1	70	0.90
Difference			8 ± 4	3.7	—3	...
Protein-rich						
Treated	6	1016	548 ± 7	53.9	331	0.60
Control	6	1016	628 ± 13	61.8	360	0.57
Difference			80 ± 15	7.9	29	0.36
Fat-rich						
Treated	5	173	94 ± 4	54.3	84	0.89
Control	5	173	106 ± 3	61.3	89	0.84
Difference			12 ± 5	7.0	5	0.42
Fasting						
Treated	6		88 ± 6			
Control	6		100 ± 3			
Difference			1[0] ± 7			

[1] ± values represent the standard errors of means or differences.

Energy metabolism

The effects of the A.P.E. on the respiratory quotients, the total heat production and the nutrient derivation of heat are presented in table 3. For purposes of comparison with the effects of extended treatment data pertaining to fasting and the stock diet (Voris et al., '42 b) are also given.

Under all treatments, fasting or feeding, the total heat production was increased in the rats treated with the A.P.E. In each case, the extra heat was produced from non-protein nutrients, and the heat derived from protein was decreased. Thus, the increase in total heat production of the treated rats resulted from an accelerated oxidation of non-protein nutrients, which was greater in magnitude than a simultaneous diminution in protein katabolism.

In the fasting period the control rats produced 21%, and the treated rats 14%, of their total heat from protein. On the stock diet the proportionate derivation of heat from protein and non-protein nutrients was almost identical with that of fast. During the feeding of the stock diet the control rats produced 78%, and the treated rats 86%, of their total heat from carbohydrate, while in the fasting period the same energy quotas were derived from fat. The non-protein respiratory quotients indicated that the control rats on the stock diet were synthesizing fat while the treated rats were oxidizing extra carbohydrate. Computation of the glucose equivalent indicated that the treated rats were oxidizing about 1.5 times as much extra carbohydrate as the control rats were synthesizing into fat. The difference in the R. Q.'s was not highly significant (odds 20 to 1 against difference occurring by chance alone) so that the computation does not merit emphasis if considered alone. However, the same relationship was found on the carbohydrate-rich diet for which the difference in R. Q.'s was of the same magnitude as that for the stock diet but statistically more highly significant.

In the feeding of the carbohydrate and the fat-rich diets the protein katabolism was diminished considerably. This resulted

TABLE 3

The effect of periodic injection of anterior pituitary extract on the total heat production and the heat derived from protein, carbohydrate and fat.

TREATMENT	NUMBER OF RATS	NON-PROTEIN R. Q.	TOTAL HEAT PRODUCTION	HEAT DERIVED FROM			
				Protein	Carbohydrate	Fat	Fat synthesis
			Cal./hr.	%	%	%	%
Initial fast							
Treated	4	0.70 ± 0.01	911	14.4	0	85.6	
Control	4	0.71 ± 0.01	775	21.0	0.8	78.2	
Stock diet							
Treated	4	1.00 ± 0.03	1025	14.2	85.8	0	0
Control	4	1.05 ± 0.03	878	20.8	78.1	0	1.1
Carbohydrate-rich diet							
Treated	4	1.09 ± 0.01	1111	6.5	91.8	0	1.7
Control	4	1.14 ± 0.01	972	8.7	88.6	0	2.7
Protein-rich diet							
Treated	4	0.91 ± 0.03	1224	49.4	34.9	15.7	0
Control	4	1.02 ± 0.03	1114	61.8	37.9	0	0.3
Fat-rich diet							
Treated	6	0.79 ± 0.01	1397	7.8	28.1	64.1	0
Control	6	0.79 ± 0.00	1239	9.7	28.6	61.7	0

from the decreased intake of dietary protein rather than from a sparing of protein katabolism by the non-protein nutrients (table 2). However, the protein sparing effect of the A.P.E. was still in evidence, and the difference, although not as conspicuous in these periods as in the others, was highly significant statistically.

The protein sparing effect of the A.P.E. was naturally most conspicuous in the feeding of the protein-rich diet. In this period the control rats produced 62%, and the treated rats 49%, of their total heat from protein. The heat of carbohydrate oxidation was about equal for the control and the treated rats, so that the extra heat produced by the latter was derived mostly from fat.

TABLE 4

Average weights and gains of rats during 2 weeks on the various diets. Treated rats (T) were injected with A.P.E. for 3 days prior to each period.

C = control pairmates.

	TYPE OF DIET								OVERALL	
	Stock		Carbohydrate-rich		Protein-rich		Fat-rich			
	T	C	T	C	T	C	T	C	T	C
Final wt., gm.	150	151	170	176	214	216	236	240	236	240
Initial wt., gm.	106	106	150	151	170	176	214	216	106	106
Gain	44	45	20	25	44	40	22	24	130	134

On the fat-rich diet the extra heat of the treated rats was derived from both carbohydrate and fat, but the latter contributed about four times as much energy as the former in the increased metabolism.

The weights and gains of the rats during the various dietary treatments are presented in table 4. The A.P.E. was administered for 3 days prior to the start of each dietary treatment, this being also the last 3 days of the prevailing treatment. With this short injection period the growth principle of the A.P.E. evidently did not affect body weight (Voris et al., '42 a), so that the results of table 4 are significant only insofar as they represent the carry-over of the A.P.E. as affecting

the compromise between the conservation of protein and the utilization of extra dietary energy for the increased metabolism. The rats treated with the A.P.E. showed excess gain over the controls only on the protein-rich diet. The difference was not statistically significant, but assumed a significance in comparison with the results on the carbohydrate and the fat-rich diets in which the weight differences favoring the control rats were statistically significant.

DISCUSSION

The results presented herein confirm and extend those presented by Voris et al. ('42 b) showing that the saline A.P.E. used in these investigations spared protein but increased the oxidation of non-protein nutrients. Also the conclusion of these authors that the increased heat production of the treated rats was the result of a true stimulation of cellular metabolism, and not the accelerated oxidation of a particular nutrient, is well supported by the present results.

The energy required for the accelerated metabolism was derived from whatever nutrient was most available at the moment. Carbohydrate appeared to be the preferred source of energy for immediate metabolic purposes.

Protein was preferentially katabolized only when the supply exceeded the body capacity for retention. Under such a condition protein may spare the oxidation of carbohydrate or fat in the same way that it is spared when the latter nutrients are in excess.

Fat was oxidized readily when its presence in the diet overbalanced that of carbohydrate and protein, but the oxidation of carbohydrate was not entirely excluded by the plethora of fat as the oxidation of fat was excluded when dietary carbohydrate was sufficient.

These observations merely represent well-recognized metabolic principles: carbohydrate is the preferred source of energy for metabolism; protein is necessary for the maintenance of cellular structure, and incidentally provides a potential source of energy; while fat is conserved under

ordinary conditions as the most efficient form of energy reserve for emergency use.

The principle of the anterior pituitary which depresses carbohydrate oxidation and increases carbohydrate reserves (Fisher, Russell and Cori, '36; Meyer, Wade and Cori, '37; Russell, '38 a) was not in evidence in the A.P.E. used in the present investigation. Russell ('38 b) has shown that the principle is not associated with the growth, adrenotropic, gonadotropic, lactogenic, or thyrotropic factors of the anterior pituitary. Greaves, Freiberg and Johns ('40) have associated the R. Q. reducing factor with the ketogenic principle.

The investigations describing the glycostatic action of the anterior pituitary have dealt with the immediate physiological response of rats to the A.P.E. with respect to a single nutrient (glucose). Under the conditions imposed the animals were in a state of physiological readjustment and the metabolic measurement could well represent the adjustmental processes rather than the regulated processes of normal nutrition. The negative effects obtained after 20 days' treatment with the A.P.E. (Russell, '38 a) may represent the latter condition rather than a refractory reaction to continued treatment.

The standardized feeding technic and the 3-day treatment employed in the present study avoided the immediate adjustmental responses to the A.P.E., as well as protein plethora, and the antagonistic effects encountered with extended treatment (Voris et al., '42 b).

Recently Gaebler and Robinson ('42) have presented further evidence that the growth preparation of the anterior pituitary stimulates the production or mobilization of insulin. However, insulin alone does not induce N storage in normal animals, nor does A.P.E. alone induce N storage in depancreatized dogs receiving insulin. While the appearance of the protein sparing or diabetogenic effects of an A.P.E. may depend on the capacity of the pancreas to produce insulin, it would seem that the effects are less specific than as suggested by Gaebler and Robinson ('42) in view of the results reported in this paper. It seems more probable that all regulatory agents con-

cerned in the maintenance of homeostasis are affected, and that the net result will depend on the capacity of the various organs producing the regulatory agents to meet the demands made upon them.

SUMMARY

The effects of limited treatment (3 days) of rats with saline anterior pituitary extract have been studied with respect to the disposition of the nutrient energy of a balanced diet, and of diets characterized by excess of protein, carbohydrate and fat. Measurements of the energy metabolism and the secretion of urinary N and C were made under each of the dietary treatments. A standardized feeding method for the treated and the control rats was used to assure experimental uniformity and nutritional adjustment to the diets prior to the metabolism measurements.

Under all dietary conditions the A.P.E. decreased the katabolism of protein, but stimulated the oxidation of non-protein nutrients. The latter effect overbalanced the former, so that the total heat production was increased. The extra heat resulted from a true stimulation of cellular metabolism, and not from the accelerated oxidation of any particular nutrient.

LITERATURE CITED

FISHER, R. E., J. A. RUSSELL AND C. F. CORI 1936 Glycogen disappearance and carbohydrate oxidation in hypophysectomized rats. J. Biol. Chem., vol. 115, p. 627.

FORBES, E. B., M. KRISS AND R. C. MILLER 1934 The energy metabolism of the albino rat in relation to the plane of nutrition. J. Nutrition, vol. 8, p. 535.

GAEBLER, O. H., AND A. R. ROBINSON 1942 Effects of the pancreas and the adrenals upon production of nitrogen storage with pituitary preparations. Endocrin., vol. 30, p. 627.

GREAVES, J. D., I. K. FREIBERG AND H. E. JOHNS 1940 Preparation and assay of anterior pituitary fractions rich in ketogenic and respiratory quotient-reducing substances. J. Biol. Chem., vol. 133, p. 243.

KRISS, M. 1938 The specific dynamic effects of proteins when added in different amounts to a maintenance ration. J. Nutrition, vol. 15, p. 565.

KRISS, M., AND L. VORIS 1937 A further contribution to the derivation of factors for computing the gaseous exchange and the heat production in the metabolism of proteins. J. Nutrition, vol. 14, p. 215.

MEYER, H. S., L. J. WADE AND C. F. CORI 1937 Influence of extracts of anterior lobe of pituitary on glucose oxidation and glycogen storage. Proc. Soc. Exp. Biol. Med., vol. 36, p. 346.

RUSSELL, J. A. 1938 a The effects of hypophysectomy and of anterior pituitary extracts on the disposition of fed carbohydrate in rats. Am. J. Physiol., vol. 121, p. 755.

———— 1938 b The anterior pituitary factor which maintains muscle glycogen in fasted hypophysectomized rats. Endocrin., vol. 22, p. 80.

VORIS, L., M. KRISS, L. F. MARCY AND R. S. BOWMAN 1942 a Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. I. Sexual differences in appetite, growth and organ weights. J. Nutrition, vol. 24, p. 469.

———— 1942 b Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. II. Protein and energy metabolism. J. Nutrition, vol. 24, p. 481.

THE METABOLISM OF ARGININE AND LEUCINE WITH SPECIAL REFERENCE TO RESPIRATORY EXCHANGE AND HEAT PRODUCTION[1]

MAX KRISS AND ROBERT S. BOWMAN[2]
Institute of Animal Nutrition, The Pennsylvania State College, State College

(Received for publication June 13, 1942)

The purpose of this investigation was to add to the existing determinations of the dynamic effects of amino acids of different groups by the particular procedure employed, that is, by difference between values for heat production from established nutritive regimens, with the metabolism of energy equilibrium as the base value.

The series of such determinations made at this laboratory, including those presented in an earlier paper (Kriss, '41) and those now here reported, represent the following kinds and classes of amino acids: monoamino monocarboxylic acids: glycine, dl-alanine, l-leucine and l-tyrosine; monoamino dicarboxylic acids: d-glutamic acid, l-aspartic acid and its half-amide l-asparagin; diamino monocarboxylic acid: arginine. Among these seven compounds only leucine and arginine, the subjects of the present study, are essential in the nutrition of the rat.

The dynamic effect of any amino acid, by whatever method determined, is in all probability affected by the other nutrients with which it is metabolized, since it would never be metabolized alone. This fact should be kept in mind, therefore, in

[1] Authorized for publication on June 8, 1942, as paper no. 1106 in the Journal series of the Pennsylvania Agricultural Experiment Station.

[2] The experimental work here reported was performed by the late Max Kriss, with the collaboration of Robert S. Bowman, this paper having been written by E. B. Forbes.

comparing dynamic effects determined by procedures implying the metabolism of fast as the base value with determinations made, as in the present investigation, with reference to the metabolism of nutritive equilibrium.

It is the authors' understanding that the advantage of employing the heat production of maintenance as the base value is that dynamic effects so determined represent conditions of practical nutrition, while dynamic effects determined with the heat production of any submaintenance nutritive status are invalidated by an assumed dynamic effect of the body nutrients katabolized — a point of view that has been stressed in several papers from this Institute since 1928 (Forbes and Swift, '41 a, '41 b; Kriss, '41).

In the present investigation the conditions were such that the dynamic effects of the amino acids, as observed, applied to the use of these acids for heat production alone since the amino acids were almost completely absorbed, and after absorption were completely eliminated in the urine.

DETAILS OF EXPERIMENTATION

This experiment was conducted during the months of October and November, 1940.

The subjects were twelve, growing, male, albino rats, six of which (nos. 61 to 66, inclusive) were used to determine the dynamic effect of arginine monohydrochloride, and six others (nos. 67 to 72, inclusive) for a similar study of leucine.

The final results for both groups, however, represent only five individuals, since rat no. 65 was eliminated because of diarrhea, and rat no. 70 was accidentally suffocated during the final respiration experiment.

The basal diet was 9 gm. per day of a nutritively complete commercial feed[3] 90% and butterfat 10%, the rats being fed twice a day, at 8:00 A.M. and 8:00 P.M. The butterfat was included for its contribution to the palatability of the diet.

The average initial body weights of the arginine and the leucine rats were 220 and 200.5 gm., respectively, and their

[3] Purina dog chow.

average weights before the final period of fast were 238 and 226.7 gm., respectively. The basal diet, therefore, provided slightly more than maintenance requirements, as intended.

The quantities of amino acids fed daily as supplements were 1.14 gm. of arginine monohydrochloride and 1.18 gm. of l-leucine, these quantities providing 10.7% and 15.2%, respectively, of the gross energy, and 50.5% and 29.4%, respectively, of the nitrogen of the entire diets in which they were fed.

Dynamic effects were determined by difference between the heat production from the basal diet and from the same plus an amino acid.

Respiration measurements, by the open-circuit Haldane procedure, began at 8:30 A.M. and continued for 7 hours.

A representative schedule of experimentation is that of rat no. 61, as follows: basal diet, October 14th–October 28th; collection of excreta, October 21st–October 28th; respiration measurement, October 28th; supplemented diet, basal plus arginine, October 28th–November 6th; collection of excreta, November 1st–November 6th; respiration measurement, November 6th; basal diet, November 6th–November 12th; respiration measurement, November 12th; fast, November 18th–19th; respiration measurement November 19th.

The alimentary fill was determined on all rats on November 27th; and the urines and the feces of the similarly treated rats were composited for analysis, the elimination of excretory constituents of the individual animals being computed from the individual quantities of excreta eliminated and the analyses of the composite samples.

The metabolism of protein and of amino acids was accounted for in the computation of the heat production in the feeding periods on the basis of the urinary nitrogen excretions and the respiration and energy factors reported by Kriss ('39) and Kriss and Marcy ('40).

The usual practice at this laboratory of computing the heat production, for comparative purposes, to a basis of uniform empty body weight was continued in the present study.

DISCUSSION OF RESULTS

The analysis of the diets is given in table 1, and that of the urines in table 2; the data for daily income, feces, urine and metabolizability of the amino acids are set forth in table 3.

These data signify that the energy and the carbon of the arginine monohydrochloride were not so well absorbed as those of the leucine; that the energy and the carbon intakes in arginine monohydrochloride were more largely eliminated in the urine than were those in leucine; that approximately all of the nitrogen of the arginine monohydrochloride and of the leucine was absorbed, metabolized, and eliminated in the urine; and that of the energy of the arginine monohydrochloride and the leucine, 64.8% and 90.0%, respectively, was metabolized.

TABLE 1

Analysis of basal ration and amino acid supplements.

MATERIAL	MOISTURE	NITROGEN	CARBON	ENERGY
	%	%	%	*cal./gm.*
Basal ration	8.39	3.25	43.55	4650
Arginine monohydrochloride	0.10	26.21	33.92	4416
l-Leucine	0.11	10.30	53.90	6369

Since protein utilization was not increased by the ingestion of these two essential amino acids, it is clear that the protein requirements of the experimental subjects were satisfied by the basal diet. The heat increments of the amino acids as observed, therefore, were unaffected by growth.

The basis on which the factors were determined for computing the respiratory exchange and the heat production in the metabolism of arginine and leucine is set forth in table 4. These values refer to moisture-free leucine, and to the pure arginine in the arginine monohydrochloride.

Values for the hourly heat production of the rats per 200 gm. of empty body weight, and for the increases in heat production due to the amino acids, on the absolute basis and

TABLE 2

Urinary analysis.

RAT NO.	BASAL RATION				BASAL RATION PLUS 1.14 GM. ARGININE MONOHYDROCHLORIDE				DIFFERENCES DUE TO AMINO ACIDS				
	Nitrogen per day	Carbon per day	C : N ratio	Energy per day	Nitrogen per day	Carbon per day	C : N ratio	Energy per day	Nitrogen per day	Carbon per day	C : N ratio	Energy per day	ENERGY : N RATIO
	mg.	*mg.*		*cal.*	*mg.*	*mg.*		*cal.*	*mg.*	*mg.*		*cal.*	
61	138	128	0.93		426	229	0.54		288	101	0.35		
62	130	127	0.98		431	235	0.55		301	108	0.36		
63	128	124	0.97		426	237	0.56		298	113	0.38		
64	132	117	0.89		414	231	0.56		282	114	0.40		
66	138	131	0.95		440	245	0.56		302	114	0.38		
Average	133	125	0.94	1321	427	235	0.55	2725	294	110	0.37	1404	4.78
					BASAL RATION PLUS 1.18 GM. LEUCINE								
67	140	123	0.88		268	178	0.66		128	55	0.43		
68	136	123	0.90		268	184	0.69		132	61	0.46		
69	133	124	0.93		257	185	0.72		124	61	0.49		
70	135	129	0.96		248	182	0.73		113	53	0.47		
71	158	140	0.89		266	192	0.72		108	52	0.48		
72	153	132	0.86		262	185	0.71		109	53	0.49		
Average	143	129	0.90	1371	262	184	0.70	2119	119	56	0.47	748	6.29

as related to the metabolizable energy of the amino acids, are given in table 5.

On either the former or the latter basis these amino acids unquestionably manifested dynamic effects, but these effects were more variable, as among the individual subjects, than were similar values derived in the earlier studies of similar character.

TABLE 3

Metabolizability of amino acids.

	ARGININE MONO-HYDROCHLORIDE 1.14 GM.	LEUCINE 1.18 GM.
Income		
Energy, cal.	5034	7515
Carbon, mg.	387	636
Nitrogen, mg.	299	121
Outgo		
Feces		
Energy, cal.	369	–
Carbon, mg.	23	–
Nitrogen, mg.	5	2
Urine		
Energy, cal.	1404	748
Carbon, mg.	110	56
Total nitrogen, mg.	294	119
Unmetabolized amino acid N, mg.[1]	0	1
Unmetabolized amino acid N, mg.[2]	0	2
Metabolized amino acid N, mg.[1]	294	118
Metabolized amino acid N, mg.[2]	294	117
Metabolized amino acid N, %[1]	100	99
Metabolized amino acid N, %[2]	100	98
Metabolizable		
Energy, cal.	3261	6767
Carbon, mg.	254	580
Energy, %	64.8	90.0
Carbon, %	65.6	91.2

[1] Computed on the basis of C: N ratio.

[2] Computed on the basis of energy: N ratio.

TABLE 4

Determination of factors for computing the respiratory exchange and the heat production in the metabolism of arginine and leucine.

	ARGININE	LEUCINE
Intake, computed per 100 gm. of pure amino acids		
Energy, Cal.	534.6	637.6
Nitrogen, gm.	32.16	10.68
Carbon, gm.	41.36	54.94
Hydrogen, gm.	8.10	9.99
Oxygen, gm.	18.37	24.40
Outgo		
Feces		
Energy, Cal.	8.8	
Nitrogen, gm.	0.53	
Carbon, gm.	0.68	
Hydrogen, gm.	0.13	
Oxygen, gm.	0.30	
Urine		
Energy, Cal.	151.0	67.1
Total nitrogen, gm.	31.63	10.68
Unmetabolized amino acid N, gm.	0.00	0.13
Metabolized N, gm.	31.63 [1]	10.55
Carbon, gm.	11.83	5.03
Hydrogen, gm.	4.80	1.64
Oxygen, gm.	15.80	6.33
Metabolized		
Energy, Cal.	374.8	570.5
Carbon, gm.	28.85	49.91
Hydrogen, gm.	3.17	8.35
Oxygen, gm.	2.27	18.07
Intramolecular H_2O		
Hydrogen, gm.	0.28	2.26
Oxygen, gm.	2.27	18.07
Carbon oxidized to CO_2, gm.	28.85	49.91
Hydrogen oxidized to H_2O, gm.	2.89	6.09
CO_2 produced, gm.	105.78	183.00
O_2 required to oxidize C, gm.	76.93	133.09
O_2 required to oxidize H, gm.	23.12	48.72
Total O_2 required, gm.	100.05	181.81
Respiratory quotient	0.77	0.73
Liters O_2 required per gram urinary N	2.21	11.91
Liters CO_2 produced per gram urinary N	1.70	8.73
Calories metabolized per gram urinary N	11.8	53.4
Calories per liter O_2	5.35	4.48
Liters O_2 required per gram N of amino acid metabolized	2.21	12.06
Liters CO_2 produced per gram N of amino acid metabolized	1.70	8.84
Calories metabolized per gram N of amino acid metabolized	11.8	54.1

[1] Of the total urinary nitrogen 3.98 gm. were calculated as ammonia and 27.65 gm. as urea.

With rat no. 62 the arginine monohydrochloride incited heat production to an extent in excess of its own metabolizable energy.

With the remaining rats of this group the dynamic effect of arginine and leucine varied between 45.8 and 77.9%, and 19.2 and 49.3%, respectively, of their metabolizable energy. In neither case does it seem justified to assume that the true value is a physiological constant. The dynamic effects, therefore, are not averaged.

TABLE 5

Hourly heat production of rats per 200 gm. of empty weight and increases in heat production due to the amino acids.

RAT NO.	BASAL DIET			BASAL DIET	INCREASE IN HEAT PRODUCTION DUE TO AMINO ACID	
	Initial period	Final period	Average		Total	Per cent of metabolizable energy
	cal.	*cal.*	*cal.*	*+ 1.14 gm. arginine*	*cal.*	
61	941	995	968	1081	113	77.9
62	971	1056	1014	1191	177	137.2
63	1014	957	986	1054	68	50.7
64	964	954	959	1040	81	63.3
66	978	999	989	1054	65	45.8
Average	974	992	983	1084		
				+ 1.18 gm. leucine		
67	1087	948	1018	1156	138	49.3
68	944	951	948	1025	77	27.6
69	1068	998	1033	1087	54	19.2
71	987	920	954	1020	66	23.2
72	1049	969	1009	1100	91	31.9
Average	1027	957	992	1078		

Since the variation among the absolute individual values was similar to that among the values referred to the average metabolizable energy, it is necessary to ascribe this variation primarily to two factors, (1) individual response of the experimental subjects to the associative effect of the basal diet and the amino acid supplements, and (2) the inevitable errors of experimental work, which in these cases were referred, by

the difference method of determination of the heat increments, to the relatively small proportions of these amino acids (10.7% and 15.2%, respectively, of the gross energy of the diets) that the rats were able to take.

The dynamic effects observed were not constant enough to serve as a basis of close comparison with the dynamic effects of other amino acids.

SUMMARY

With albino rats as subjects, the dynamic effects of arginine monohydrochloride and leucine were determined by superimposition upon a nutritively complete basal diet.

Virtually all of the nitrogen of these amino acids was absorbed, metabolized, and eliminated in the urine; and of the energy of these two compounds, 64.8% and 90.0%, respectively, was metabolized.

The dynamic effects of arginine and leucine varied between 45.8% and 137.2%, and 19.2% and 49.3%, respectively, of their metabolizable energy. In neither case does it seem justified to assume that the true value is a physiological constant.

LITERATURE CITED

FORBES, E. B., AND R. W. SWIFT 1941 a The minimum base value of heat production in animals. Science, vol. 93, p. 623.

FORBES, E. B., AND R. W. SWIFT, WITH COLLABORATION 1941 b The minimum base value of heat production in animals. A research in the energy metabolism of cattle. Penna. Agr. Expt. Sta., Bul. 415.

KRISS, MAX 1939 A comparative study of the metabolism of certain amino acids, with special reference to respiratory exchange and heat production. J. Nutrition, vol. 17, no. 1, p. 1.

——— 1941 The specific dynamic effects of amino acids and their bearing on the causes of specific dynamic effects of proteins. J. Nutrition, vol. 21, no. 3, p. 257.

KRISS, MAX, AND LAWSON F. MARCY 1940 The metabolism of tyrosine, aspartic acid and asparagine, with special reference to respiratory exchange and heat production. J. Nutrition, vol. 19, no. 3, p. 297.

AN EVALUATION OF THE NUTRITIONAL STATUS OF A POPULATION GROUP IN MADRID, SPAIN, DURING THE SUMMER OF 1941 [1]

WILLIAM D. ROBINSON, JOHN H. JANNEY AND FRANCISCO GRANDE (COVIAN)

The Rockefeller Foundation Health Commission, New York, and Institute of Medical Research, Madrid

(Received for publication June 17, 1942)

This communication deals with an attempt to ascertain some facts regarding the nutritional state of a population group in Madrid, Spain, during the summer of 1941. The survey was confined to an industrial suburb of Madrid. It was made on a family basis with the sample drawn in accordance with the geographic distribution of the population, and utilized the combined methods of dietary records, clinical examination, and appropriate laboratory determinations. In all, 106 families composed of 561 individuals were examined between the end of May and the middle of September, 1941.

METHODS

The dietary records were collected on a family basis by the inventory and purchase method (Bigwood, '39), the consumption of foodstuffs by each family being determined for

[1] The studies and observations on which this report is based were conducted with the support and under the auspices of The Rockefeller Foundation Health Commission and with the collaboration of the Dirección de Sanidad of the Spanish Government. The authors are indebted to Dr. John B. Youmans for assistance in the organization of the study, and to Miss Persis Putnam for aid and advice in the statistical analysis of the data. The American Red Cross Commission to Spain furnished material assistance in the form of foodstuffs as a reward to the subjects studied. Drs. T. Solves and Juan Rof (Carballo) assisted in the clinical examinations.

7 consecutive days. Each family was visited daily, and the amount of food purchased that day was weighed, together with the refuse, if any, from the previous day. At the same time the cost of each item of food was recorded, furnishing a record of the amount spent for food by the family during the week. This family record was supplemented by the following individual records made by the investigator during the week of the family inquiry: (1) household measurements of the meal by food consumption of one adult in each family for 3 nonconsecutive days, (2) number of servings of each foodstuff eaten during the week by each child under 10 years of age, obtained by questioning and expressed in household measurements, (3) feeding since birth of all infants under 2 years of age. With families who were receiving meals from the relief agency, information was obtained from an official of the agency as to the quantity fed per person of each foodstuff used in preparing the dish served each day.

For the calculation of the dietary constituents, food tables were compiled in an attempt to approximate the content of Spanish foods, in so far as this was possible with available data. Carbohydrate, fat, protein, and caloric values were obtained from the few analyses which have been made of Spanish foodstuffs (Vazquez Sanchez, '32; Puyal and Srta. Torres, '32; Grande Covian, '39) and from European sources (Randoin, '39; Schall, '41). It was necessary to assume from analyses in other countries the values for minerals (Sherman, '37) and vitamins (Booher and Hartzler, '39; Fixsen and Roscoe, '40; Droese and Bramsell, '41). A single value for vitamin content usually had to be selected arbitrarily from a wide range of values offered by analyses in other parts of the world. This is a probable source of error particularly with respect to vitamins A and C, which are known to vary considerably with geographic and seasonal sources. No allowance has been made for losses in cooking; the cooking habits were such that this loss was probably small.

The clinical examination consisted of the usual diagnostic history and physical examination, with special attention

directed to symptoms and signs of deficiency disease and the detection of concomitant conditions which may affect the nutritional status. In addition, the cornea was examined with the slit lamp and biomicroscope for evidence of capillary invasion, roentgenograms were obtained of the hand and lower leg on all children under 10 years of age, and certain anthropometric determinations were made.

About 20 cc. of venous blood was drawn from each subject with precautions against stasis and hemolysis; 4 cc. was oxalated with potassium and ammonium oxalate in suitable proportions and quantity to prevent change in cell size. On this was determined the hemoglobin concentration, erythrocyte count, and hematocrit. The remainder was allowed to clot.

The hemoglobin was determined by the method of Evelyn ('36) for oxyhemoglobin, the readings being made with the photoelectric colorimeter and converted to grams hemoglobin per 100 cc. by using K 2.58. Frequent checks indicated the accuracy of this method under conditions of the survey to be ± 0.2 gm. per 100 cc.

The red blood cell counts were made by the usual method using Thoma diluting pipettes, shaking by hand, and counting areas in two counting chambers on each specimen. Repeated duplicate counts indicated the accuracy under survey conditions to be $\pm 150{,}000$ per cubic millimeter.

The hematocrit determinations were made in standard Wintrobe tubes, the packed red cell volumes being red after 1 hour centrifuging at 3500 r.p.m. Duplicate determinations indicated an accuracy of ± 0.5 vol. per cent.

Serum protein and serum albumin were determined by the quantitative biuret reaction (Robinson and Hogden, '40). The readings were made on the photoelectric colorimeter with a standard of preserved rabbit serum on which several macro-Kjeldahl nitrogen determinations were made. The separation of albumin and globulin was obtained by salting out with 22% sodium sulphate and filtration.

Serum ascorbic acid content was determined by the titration of a metaphosphoric acid filtrate of the serum with 2,6

dichlorophenolindophenol (Farmer and Abt, '35). The dye was standardized against fresh solutions of ascorbic acid, and redistilled water used in all reagents.

Serum carotene and vitamin A were determined by using a petroleum ether extract of alcohol-treated serum and the Carr-Price reaction, utilizing the micro-unit of the photoelectric colorimeter (May et al., '40). The carotene readings were made with a 420 filter, but a reference curve derived with this filter for beta-carotene was used as a standard. The vitamin A content was calculated by using the following arbitrary constants:

L_{420}–0.11 L_{430} to correct for blue color contributed by carotenoids

0.41 to convert $L^{1\%}_{1\text{ cm.}}$ to $E^{1\%}_{1\text{ cm.}}$

2100 to convert $E^{1\%}_{1\text{ cm.}}$ to International Units.

The phosphatase activity and the inorganic phosphorus content of the sera of children under 6 years of age were determined by the methods of Bodansky ('33) and Fiske and Subbarow ('25), respectively.

CHARACTERISTICS OF SAMPLE

The district in which the subjects of this study lived, Puente de Vallecas, is on the outskirts of Madrid, directly continuous with the city itself and extending out into the arid plain on which the capital is set. While some of the inhabitants go out to work by the day in the fields, there was no significant production of food in the area for home consumption. The chief industries are the railroad repair shops and the tanneries. Most of the subjects were unskilled or semiskilled laborers or were engaged in small business enterprises. Thirty-two of the 106 families studied were receiving a substantial amount of food from the official relief agency, Auxilio Social. For most of these, this consisted of one serving of thick soup daily for each member of the family; in a few families some of the children between the ages of 5 and 12 years were getting two meals a day in the dining rooms of this agency.

During the period of the survey this agency was feeding about 12,500 persons a day, some 10,600 from the soup kitchens and 1,850 in children's dining rooms.

The census of December, 1940, lists the population of the district at 58,878; this figure is estimated to be about 20% below the actual population level. Therefore the sample represents less than 1% of the total population of the district, and is weighted with a larger proportion of families receiving relief assistance. The sample does conform fairly well to the density of population in the geographic subdivisions of the area.

TABLE 1

Age and sex distribution of subjects.

SEX	AGE IN MONTHS	AGE IN YEARS								
	2–24	2–5	6–12	13–19	20–29	30–39	40–49	50–59	60 and over	Total
Males	17	30	83	42	14	20	33	10	—	249
Females	24	35	83	40	28	46	36	8	12	312
Total	41	65	166	82	42	66	69	18	12	561

Table 1 presents the distribution by age and sex of the subjects studied. It should be noted that nearly one-half of the total were under 13 years of age and that there were fewer men than women between the ages of 20 and 40. No data were available on the age and sex distribution of the general population.

Inadvertent selection was undoubtedly operating to some extent, as in all surveys of this nature, since only persons who will cooperate in the diet inquiry, clinical examination, and bloodletting can be studied. The incentive offered, 1 kg. of flour or two cans of evaporated milk for each person completing the entire study, might have been expected to bring in families with more limited food supplies. Actually most of the refusals occurred among persons who were unable or unwilling to understand the purpose of the study, and these were more frequent among the poorer families. There were nine additional families on whom the dietary records were

completed who subsequently refused the medical examination; their dietary intake and expenditure for food fall in the range of the 106 families completely studied.

FINDINGS

The data collected are presented in tables which have been condensed from those used in the statistical calculations by decreasing the number of intervals and by combining age and sex groups when analysis has shown no significant differences among them. The mean and standard deviation, together with the standard error of each, are given for each subdivision. Median values are added to those tables where the distribution of values is "skew" rather than "normal."

Diet records

The dietary intakes have been calculated almost entirely from the family consumption records obtained by the inventory and purchase method, using the individual records chiefly to check on the equality of distribution of food consumption within the family. While there are shortcomings in such use of family consumption records, the circumstances under which this survey was conducted made them definitely the most accurate dietary information that was obtained. They were collected by the field workers in terms of actual weight, eliminating variability due to degree of cooperation and interest on the part of the housewife. Few of the families had any stores of foodstuffs at either the beginning or the end of the week, and none produced at home a significant amount of the food consumed. The amount of food eaten away from home was negligible.

To express the family intake in terms of individual consumption, the average daily intake of each dietary constituent for the entire family was computed, and that value for calories, carbohydrate, fat, total protein, and animal protein was divided by the number of adult male consumption units in the family. The latter were determined by using the caloric

coefficients of Bigwood ('39). Adjustments were made when indicated by the records of individual consumption; this was most often necessary with respect to milk used in supplementary feedings of nursing infants.

Therefore the values in table 2 represent the average daily intake of the family in terms of an adult male of average size performing moderate work, and the relative adequacy of

TABLE 2

Daily dietary intake of families in terms of adult male consumption unit.

CALORIES		CARBOHYDRATE		FAT		TOTAL PROTEIN		ANIMAL PROTEIN	
per day	*families*	*gm./day*	*families*	*gm./day*	*families*	*gm./day*	*families*	*gm./day*	*families*
Below 750	4	Below 100	3	5–25	30	Below 25	4	0–7.5	19
750–1250	28	100–200	43	25–50	39	25–50	29	7.5–20	37
1250–1750	35	200–300	34	50–75	21	50–75	36	20–32.5	29
1750–2250	22	300–400	15	75–100	10	75–100	23	32.5–45	8
2250–2750	7	400–500	4	Above 100	3	100–120	2	45–57.5	4
Above 2750	6	Above 500	3			Above 120	2	57.5–70	4
								Above 70	2
Total	102		102		103		103		103
Mean	1602 ± 61		238 ± 10		42 ± 3.0		66 ± 2.7		22 ± 1.7
Standard deviation	616 ± 43		105 ± 7		30 ± 2.1		27 ± 1.9		17 ± 1.2
Median	1543		218		37		63		18

the diet for each individual is obtained by comparing the given value with the requirement of such an adult male. This procedure makes it possible to reduce to a common basis the dietary intakes of families differing in age and sex composition, and so differing in caloric and protein requirements. The protein requirement is closely related to that for calories, being dependent on age and weight, and Bigwood's protein

TABLE 3

Dietary intake of families per person per day.

CALCIUM		PHOSPHORUS		IRON		VITAMIN A		THIAMINE		RIBOFLAVIN		ASCORBIC ACID	
gm. per head	*no. of families*	*gm. per head*	*no. of families*	*mg. per head*	*no. of families*	*I.U. per head*	*no. of families*	*mg. per head*	*no. of families*	*mg. per head*	*no. of families*	*mg. per head*	*no. of families*
.075–.150	17	.250–.500	8	3–6	6	Below 1500	17	.250–.500	12	.100–.250	12	2–20	11
.150–.300	44	.500–.750	17	6–9	27	1500–3000	35	.500–.750	35	.250–.500	46	20–40	34
.300–.450	26	.750–1.000	29	9–12	26	3000–4500	17	.750–1.000	30	.500–.750	25	40–60	35
.450–.600	8	1.000–1.250	20	12–15	27	4500–6000	13	1.000–1.250	15	.750–1.000	11	60–80	13
.600–.750	6	1.250–1.500	19	15–18	8	6000–7500	7	1.250–1.500	8	1.000–1.250	6	80–100	4
Above .750	2	1.500–1.750	6	18–21	5	7500–9000	7	Above 1.500	2	Above 1.250	3	100–120	4
		1.750–2.000	4	Above 21	4	Above 9000	7					Above 120	2
Total families	103		103		103		103		102		103		103
Mean	.301 ± .018		1.021 ± .036		11.4 ± .43		3852 ± 273		.820 ± .030		.542 ± .029		47.4 ± 2.8
Standard deviation	.181 ± .013		.366 ± .026		4.3 ± .30		2767 ± 193		.304 ± .021		.294 ± .020		28.3 ± 2.0
Median	.256		.983		11.3		2875		.794		.475		44.2

coefficients correspond almost exactly with his calorie coefficients, so no significant error is introduced by not using a separate system of coefficients for protein.

With respect to mineral and vitamin intake, no satisfactory system of coefficients is available and the requirements show no constant change with age and sex. Accordingly the intake of these constituents (table 3) is expressed as daily intake per person, obtained by dividing the daily average consumption by the number of persons in the family.

The foods of chief importance in determining the caloric intake were bread, which frequently supplied more than one-half of the total calories, olive oil, potatoes, and sometimes rice; occasionally a fresh vegetable was eaten in such enormous quantities that it made a significant contribution to the caloric total. The amount of olive oil consumed virtually determined the fat intake. Animal protein was supplied almost entirely by fish, with milk making an occasional small contribution; meat, eggs, and cheese were practically unknown. Vegetable protein was derived largely from bread, fresh or dried beans, potatoes and garbanzos. The bread was a coarse, dark brown, whole grain product (chiefly wheat) which also contributed significantly to the total mineral intake, especially of iron and phosphorus, and was of paramount importance in determining the thiamine intake. Vitamin A was almost entirely in the form of carotene from fresh vegetables, mainly string beans, lettuce, tomatoes, and chard. These same vegetables plus potatoes and peppers largely determined the ascorbic acid intake; fruit was out of the financial reach of these people. The nicotinic acid content of the diets has not been calculated, as reliable values are available for only a few of the foods consumed. It is probable that the pellagra-preventive value of these diets is limited, and provided chiefly by the fish and green vegetables.

Table 4 indicates the derivation of calories from the three primary food constituents. There is a tendency for carbohydrates to contribute a relatively high proportion of the total energy. There was no definite correlation between the

total calories in the diet and the proportion contributed by any constituent; some of the families in the higher caloric levels got most of the additional calories from carbohydrates, others from a higher intake of olive oil. The proportion of calories derived from protein was relatively constant.

TABLE 4

Daily dietary intake of families: derivation of calories.

PER CENT OF TOTAL CALORIES	NUMBER OF FAMILIES DERIVING STATED PER CENT OF CALORIES FROM:		
	Carbohydrate	Fat	Protein
5	—	4	—
10	—	22	11
15	—	19	59
20	—	9	26
25	—	10	5
30	1	9	—
35	1	10	1
40	5	11	—
45	9	6	—
50	13	2	—
55	13	—	—
60	12	—	—
65	9	—	—
70	25	—	—
75	10	—	—
80	3	—	—
85	1	—	—
Total families	102	102	102
Mean per cent of total calories	60.4 ± 1.16	23.4 ± 1.24	16.4 ± 0.40
Standard deviation	11.8 ± 0.82	12.5 ± 0.87	4.0 ± 0.28

In considering the dietary requirements of this group, certain observations should be mentioned. While no attempt has been made to calculate the energy requirements of each individual, an approximation of the mean and range can be made by using the recorded weights and a rough estimate of the level of activity (table 5). It appears that the figure of

TABLE 5

Energy requirements based on observed mean height and weight.[1]

NUMBER PERSONS AGE 18–49 YEARS		HEIGHT IN CUBIC METERS		WEIGHT IN KILOGRAMS	
		Mean	Standard deviation	Mean	Standard deviation
Males	70	162 ± .7	6.2 ± .5	54.1 ± .7	6.1 ± .5
Females	111	151 ± .6	5.9 ± .4	46.6 ± .6	6.5 ± .4

	HOURS	CALORIES/ KILOGRAMS PER HOUR	CALORIES REQUIRED		
			Mean weight -2σ	Mean weight	Mean weight $+2\sigma$
Males					
Sleeping	8	0.93	312	402	493
At rest	4	1.43	240	309	379
Light exercise	6	2.43	612	789	965
Active exercise	6	4.14	1043	1344	1644
Total calories per day			2207	2844	3481
Females					
Sleeping	8	0.93	251	347	443
At rest	4	1.43	193	267	340
Light exercise	8	2.43	655	906	1157
Active exercise	4	4.14	558	772	985
Total calories per day			1657	2292	2925

[1] Sherman and Langford ('40).

2,800 calories is justified as the mean energy requirement of the adult males in this study, and should be used for comparison with the values for caloric intake in table 2. This estimated mean requirement also furnishes some justification for the use of Bigwood's coefficients in the calculation of this table.

The data on body weight also furnish a basis for estimating the protein requirement. If the usual figures of $\frac{2}{3}$ to 1 gm. of protein for each kilogram of body weight are used, the absolute values for total protein intake are in the range of adequacy for most of the families. However, the relative adequacy of the protein ration is impaired by two other characteristics of the diet: the relatively low proportion of protein from animal sources in the majority of the families,

which opens up the possibility of qualitative protein deficiency, and the almost uniformly low caloric intake, which is known to increase the protein requirement.

This low caloric intake undoubtedly exerts a protective action as far as vitamin and possibly mineral requirements are concerned. The only constituent concerning which this relationship is well enough understood to permit quantitative expression is the association of the thiamine requirement with the intake of nonfat calories (Williams and Spies, '38). The mean ratio of thiamine to nonfat calories for the families studied was $0.70 \pm .018$ with a standard deviation of $0.18 \pm .013$. For none of the families was the ratio 0.3 or less, the level at which clinical beriberi is stated to appear.

In general, the dietary intake of this group may be characterized as far below maintenance in caloric value and calcium content, and relatively inadequate in protein of animal origin. It appears relatively adequate in iron, thiamine, ascorbic acid, and probably phosphorus. Vitamin A and riboflavin levels are low and may well be below minimum maintenance requirements.

The question of how long these people had subsisted on such a regime is of paramount importance if the dietary data are to be correlated with the clinical and laboratory data. It was impossible to obtain reliable individual information on this point. In general, it appeared that these people were eating as good a diet from the qualitative point of view during the survey period as at any time during the past $2\frac{1}{2}$ to 4 years, although the quantity may have been greater and certainly has been less at intervals in the past. It should be borne in mind that these data were collected during the summer months.

Clinical examination

The following diagnoses of organic disease were made on the basis of the history and physical examination:

	NUMBER OF PERSONS
Definite tuberculosis	13
Suspected tuberculosis	17
Syphilis	12
Rheumatic heart disease	7
Gall-bladder disease	6
Hypertension	4
Progressive muscular dystrophy	3 (all brothers)
Malaria	2
Bacterial endocarditis, hepatic cirrhosis, and perinephric abscess	1 each

Among the women in the reproductive age period, two were pregnant and twenty-nine were lactating. The data on persons with active infections or metabolic disorders are not included in the tables of clinical and laboratory findings; with respect to other conditions, findings which may be due to the concomitant disease or state are excluded.

The incidence of classical deficiency disease was slight: there were two cases of definite nutritional edema, one subject with glossitis, and a chronic pellagrous dermatitis; among twenty children under 2 years of age on whom x-ray films were obtained two cases of active rickets were diagnosed. No cases of xerophthalmia, scurvy, beriberi, or specific cheilosis were seen.

The incidence of symptoms and signs which have been attributed to general undernutrition, or suggested as early manifestations of specific deficiencies, is indicated in table 6. Since many of these are not specific or their significance is controversial, the actual complaints and findings are listed. It should be borne in mind that these are subjects in whom organic disease has been excluded, in so far as this is possible, by means of clinical examination.

The most frequent item was a history of weight loss, supported by the finding of a large number with evidence thereof, chiefly in the form of scant subcutaneous tissue. A number of the children were judged to be poorly developed, and over one-third were noted to have flaring rib margins and flaccid abdominal musculature. More specific deformities of healed

TABLE 6

Incidence of symptoms and physical findings attributable to nutritional deficiency.

	AGE IN MONTHS 2–24	UNDER 13 YEARS				OVER 13 YEARS			
		2–5	6–12	Total		Males	Females	Total	
				Number	Per cent			Number	Per cent
Total persons	39	63	155	257	100	110	160	270	100
	Symptoms								
Weight loss	18	37	125	180	70	83	131	214	79
Weakness and/or ease of fatigue	4	22	102	128	50	80	133	213	29
Irritability	3	6	20	29	11	10	28	38	14
Psychic changes	—	2	4	6	2	13	32	45	17
Unexplained secondary amenorrhea	—	—	—	—	—	—	11	11	7 [1]
Night blindness	—	—	—	—	—	4	9	13	5
Paresthesias and pain in legs	—	11	68	79	31	62	113	175	65
Photophobia	—	2	15	17	7	16	30	46	17
Sore mouth	—	1	9	10	4	6	12	18	7
Gastrointestinal symptoms	—	—	11	11	4	14	25	39	14
Bleeding gums	—	1	10	11	4	8	12	20	7
Edema	—	—	2	2	1	9	24	33	12
	Physical findings								
Scant subcutaneous fat	14	19	98	131	51	47	69	116	43
Poor physical development	12	8	28	48	19	6	4	10	4
Flaring costal arch, pot belly	9	39	43	91	35	—	—	—	—
Hyperkeratosis pilaris	—	6	40	46	18	8	18	26	10
Calf muscle tenderness	—	2	25	27	10	32	49	81	30
Atrophy lingual papillae	—	3	17	20	8	10	31	41	15
Inflammation oral mucosa	—	—	—	—	—	12	13	25	9
Minor skin lesions	1	16	78	95	37	39	69	108	40
Bleeding gums	—	—	5	5	2	1	4	5	2
Edema, minimal	—	—	—	—	—	1	3	4	1
Edema, frank	—	—	—	—	—	1	1	2	1

[1] Females over 13 years of age.

rickets, such as rachitic rosary and cranial bossae, were rarely encountered. More than three-fourths of the subjects over 5 years of age complained of weakness and ease of fatigue which they claimed was a definite change from their previous status. A significant number complained of being more irritable than previously. The psychic disturbances complained of were usually apathy in the children and loss of memory in the adults. Of interest is the occurrence of secondary amenorrhea in eleven women between the ages of 18 and 36, with no other apparent physiologic or pathologic explanation.

With respect to possible vitamin A deficiency, thirteen persons complained of night blindness and seventy-two presented skin lesions with horny spines in the hair follicles. No tests of dark adaptation were made. The skin lesions were usually seen on the lateral aspect of the arms and thighs, although in a few instances they were confined to the abdomen. The condition showed a definite tendency to occur in several members of the same family.

A surprisingly large number of subjects complained of paresthesias in the extremities and pain or aching in the muscles; this was associated with the finding of calf muscle tenderness in many of these persons. In the United States, these symptoms and findings would usually be attributed to a thiamine deficiency, but there are two reasons for questioning such an interpretation in the present study. The first is the relative adequacy of the thiamine intake indicated by the dietary records. The second is concerned with observations made in Madrid during the civil war (Grande Covian and Jiménez Garcia, '40, I and II; Grande and Peraita, '41). During the food restriction incident to the siege of Madrid, over 700 cases of a severe paresthetic syndrome were studied carefully, none of which presented or developed characteristic motor or reflex changes of peripheral neuritis. The peak of occurrence of this condition was closely related to an epidemic of pellagra. The condition did not respond to thiamine, but was benefited by the administration of yeast. These experiences suggest that some other factor in the vita-

min B complex is involved. The dietary records lend support to the possibility that calcium deficiency may have been a factor in the findings in the present study.

Symptoms which have been suggested as early manifestations of a vitamin B complex deficiency of a pellagrous nature occurred in relatively few of the subjects. Little reliance could be placed on gastrointestinal complaints, because of a smoldering epidemic of infectious gastroenteritis in this area throughout the summer. Atrophy of the lingual papillae and mild inflammation of the oral mucosa were observed chiefly in adults. A large number of subjects presented minor skin lesions, such as thickening over the pressure points, loss of elasticity, scaling, etc. The circumstances under which these subjects were living (much exposure to sun and dirt, the virtual absence of soap, a high incidence of scabies and louse infestation) make it impossible to rely on such observations as manifestations of a deficiency state.

The serum ascorbic acid levels gave no grounds for attributing the infrequent complaint or finding of bleeding gums to a vitamin C deficiency.

Although thirty-five subjects gave a history of recent edema, only four cases of minimal and two cases of frank edema were encountered on examination; none of these had albuminuria or heart disease. The two cases of frank edema were definitely of nutritional origin, occurring in the father and mother of a family whose dietary record indicated a daily average intake per adult male unit of 750 calories and 33 gm. of protein, none of animal origin. The serum protein values per 100 cc. were 5.9 gm. total protein for the man, with 3.5 gm. of albumin, and 6.9 gm. total for the woman, with 3.8 gm. of albumin.

The value of the recorded body weights and measurements is impaired by the absence of such data on normal persons of Spanish stock. The weights of the subjects free from organic disease are compared with American standards for sex, height, and age in table 7. Of the 521 individuals, 332 were more than 7.5% underweight, and 213 more than 12.5% underweight.

TABLE 7

Body weight. Per cent variation from American standards[1] for height, age, and sex.

	0–24 MONTHS	2–5 YEARS	6–12 YEARS	13–19 YEARS	20–49 YEARS	OVER 49 YEARS	TOTAL
per cent							
Above +12.5	—	1	—	3	1	—	5
+12.5 to +7.5	4	—	2	4	—	—	10
+7.5 " +2.5	4	3	7	2	4	—	20
+2.5 " —2.5	3	12	16	12	15	1	59
—2.5 " —7.5	7	16	37	16	19	—	95
—7.5 "—12.5	8	15	44	16	32	4	119
—12.5 "—17.5	4	12	37	11	37	2	103
—17.5 "—22.5	3	2	10	8	24	6	53
—22.5 "—27.5	2	—	3	5	14	6	30
—27.5 "—32.5	3	—	—	—	12	4	19
Below —32.5	—	—	1	—	5	2	8
Total	38	61	157	77	163	25	521
Mean	—8.3 ±1.9	—6.8 ±.9	—9.1 ±.6	—7.6 ±1.1	—14.1 ±.8	—21.4 ±1.8	—10.7 ±.4
Standard deviation	11.5 ±1.4	7.1 ±.6	7.1 ±.4	9.9 ±.8	9.6 ±.5	9.1 ±1.3	9.6 ±.3

[1] Standards obtained from following tables:
Birth to 5 years.....R. M. Woodbury
6 to 18 years.....B. T. Baldwin and T. D. Wood
Above 18 years.....T. D. Wood

Additional evidence of general quantitative undernutrition (Benedict, '19) is seen in the tendency of the blood pressures of these subjects to be low (table 8).

The findings concerning capillary invasion of the cornea are recorded in an arbitrary descriptive classification in table 9. A number of subjects had evident conjunctival disease, usually trachoma, which may have accounted for the invasion seen. Sixteen per cent of the males and 26% of the females had findings which may be attributed to active ariboflavinosis (Sydenstricker, Sebrell, Cleckley and Kruse, '40) and an additional 12 and 10%, respectively, had findings typical of previous or disappearing deficiency. We feel that there is some uncertainty regarding the significance of invasion limited to the nasal quadrant. A higher incidence of

TABLE 8

Blood pressure.

SYSTOLIC	13–20 YEARS		21–39 YEARS		40–78 YEARS	
	Male	Female	Male	Female	Male	Female
(*mm. Hg.*)						
75– 84	1	—	—	—	—	—
85– 94	4	3	1	1	4	2
95–104	12	12	6	8	5	7
105–114	7	14	9	15	4	6
115–124	2	1	4	10	10	8
125–134	—	1	5	8	5	3
135–144	—	1	—	1	1	3
145–154	—	—	—	—	1	2
Over 154	—	—	—	—	—	5
Total	26	32	25	43	30	36
Mean	102 ± 1.8	106 ± 1.8	112 ± 2.3	114 ± 1.7	115 ± 2.8	124 ± 4.0
St. dev.	9.4 ± 1.3	10.4 ± 1.3	11.7 ± 1.7	11.4 ± 1.2	15.5 ± 2.0	24.2 ± 2.9
DIASTOLIC (*mm. Hg.*)						
45– 54	5	2	1	1	2	2
55– 64	14	11	6	6	7	2
65– 74	5	14	10	13	9	12
75– 84	2	5	7	19	12	11
85– 94	—	—	1	4	—	8
95–105	—	—	—	—	—	1
	26	32	25	43	30	36
Mean	62 ± 1.6	67 ± 1.5	70 ± 1.9	74 ± 1.4	70 ± 1.8	77 ± 1.9
St. dev.	8.3 ± 1.2	8.2 ± 1.0	9.4 ± 1.3	9.3 ± 1.0	9.6 ± 1.2	11.5 ± 1.4

capillary invasion is apparent in females of all ages; the incidence was no higher among the lactating women than among nonlactating women in the same age group, although the most severe cases were seen in the former group. While the number of persons over 50 years of age is small, there is a suggestion of lower incidence in this age group. Sydenstricker and collaborators have remarked on the possible effect of arcus senilis in preventing capillary invasion. As Youmans ('41) found in Marseille, the symptom of photophobia was of little help in predicting the slit lamp findings.

TABLE 9

Capillary invasion of the cornea.

Sex	Males						Females					
Age in years	6–12	13–20	21–49	Over 49	Total		6–12	13–20	21–49	Over 49	Total	
					Number	Per cent					Number	Per cent
Invasion												
None	39	23	33	7	102	57	21	15	38	12	86	38
Nasal quadrant only	7	2	9	1	19	11	16	7	16	2	41	18
More than nasal quadrant												
Less than 25%	9	4	8	–	21	12	6	4	11	1	22	10
More than 25%	7	8	12	1	28	16	16	14	27	2	59	26
Other eye disease	4	2	3	–	5	5	3	3	11	3	20	9
Total persons examined	66	39	65	9	179	100	62	43	103	20	228	100
Riboflavin intake	Below 0.475 mg.			Above 0.475 mg.			Below 0.475 mg.			Above 0.475 mg.		
Invasion												
None	37			50			22			48		
Nasal quadrant only	7			10			16			20		
More than nasal quadrant												
Less than 25%	9			7			14			8		
More than 25%	12			14			38			12		
Other eye disease	5			7			8			10		
Total persons under 50 years of age examined	70			88			98			98		

To investigate crudely the relationship between riboflavin intake and corneal invasion, the subjects under 50 years of age have been grouped according to riboflavin intake shown in the family diet record, the individuals being classified as they fell above or below the median value. The results are shown in table 9. The correlation between low levels of riboflavin intake and the incidence of corneal invasion seems quite definite among the females, but is not apparent among the males.

X-rays revealed active rickets in two of the twenty children between the ages of 3 to 24 months on whom the reports of the films are available. In addition, the x-ray showed definite evidence of incompletely healed rickets in one child 3 years old. No active or healed scorbutic lesions were seen by x-ray. The serum phosphatase level was definitely abnormal in only one child among the fifty-eight under the age of 6 years on whom this determination was made. This child had active rickets by x-ray and a serum phosphatase activity of 27 Bodansky units per 100 cc. of serum. No determination was made on the other child with active rickets.

The growth and developmental status of the children, as evidenced by weight, body measurements, and x-ray studies, are reported in a separate communication (Robinson, Janney and Grande Covian, '42). In general, the children included in this survey are from 1 to 3 or more years behind American children of the same age with respect to weight, height, and skeletal maturation, and there is x-ray evidence of repeated disturbances of growth of the long bones.

Laboratory determinations

The hematologic determinations and the measurements derived from them are presented in tables 10 to 12. Additional calculations of the values for the women who were lactating compared to those of nonlactating controls of the same age indicate that this condition was not a significant factor affecting the values obtained on the women during the reproductive age period.

Table 10 shows that 16% of the males and 18% of the females had hemoglobin values of less than 12 gm. per 100 cc. This does not suggest a high incidence of anemia in the population. However, in table 11 it is seen that 31% of the males and 33% of the females had red blood cell counts of less than 4,125,000, and that the values for males fail to show the normal tendency to be higher than those for females. Table 12 shows

TABLE 10

Hemoglobin.

GRAMS PER 100 CC.	MALES					FEMALES					
	2–24 months	2–12 years	13–20 years	21–60 years	Total	2–24 months	2–12 years	13–20 years	21–49 years	Over 49 years	Total
Below 10.5	—	—	—	—	—	1	—	—	1	—	2
10.5–12.0	4	21	4	6	35	6	26	—	13	2	47
12.0–13.5	6	54	17	14	91	8	56	17	44	7	132
13.5–15.0	1	20	16	30	67	1	20	20	40	2	83
15.0–16.5	—	4	2	14	20	—	3	3	4	4	14
Above 16.5	—	1	—	3	4	—	—	—	—	—	—
Total	11	100	39	67	217	16	105	40	102	15	278
Mean	12.5 ±.26	12.8 ±.11	13.4 ±.18	14.1 ±.17	13.3 ±.09	12.0 ±.28	12.7 ±.10	13.7 ±.14	13.2 ±.11	13.7 ±.34	13.5 ±.07
Standard deviation	.9 ±.18	1.1 ±.08	1.1 ±.13	1.4 ±.12	1.4 ±.07	1.1 ±.19	1.0 ±.07	0.9 ±.10	1.1 ±.08	1.3 ±.24	1.1 ±.05

TABLE 11

Red blood cell counts.

ERYTHROCYTE COUNT IN MILLIONS PER MM³.	MALES					FEMALES				
	2–24 months	2–12 years	13–20 years	Over 20 years	Total	2–24 months	2–12 years	13–20 years	Over 20 years	Total
Below 3.62	1	—	3	6	10	1	1	1	6	9
3.63–4.12	1	35	8	14	58	5	34	11	32	82
4.13–4.62	5	49	22	28	104	5	51	18	55	129
4.63–5.12	4	14	6	15	39	5	16	9	24	54
5.13–5.50	—	2	—	3	5	—	2	1	—	3
Total	11	100	39	66	216	16	104	40	117	277
Mean	4.45 ±.15	4.29 ±.04	4.28 ±.06	4.34 ±.06	4.31 ±.03	4.31 ±.10	4.29 ±.04	4.37 ±.06	4.29 ±.04	4.30 ±.02
Standard deviation	0.48 ±.10	0.38 ±.03	0.39 ±.04	0.53 ±.05	0.43 ±.02	0.38 ±.07	0.38 ±.03	0.39 ±.04	0.38 ±.03	0.38 ±.02

TABLE 12

Hematologic measurements.

Sex	Males				Females			
Age in years	0–12	13–20	Over 20	Total	0–12	13–20	Over 20	Total
Mean corpuscular volume								
cubic micra								
Below 74	1	—	—	1	—	—	—	—
74– 79	6	—	—	6	7	—	4	11
80– 85	27	5	3	35	33	2	10	45
86– 88	16	11	4	31	19	10	22	51
89– 94	41	10	22	73	38	14	40	92
95–100	13	7	18	38	16	6	25	47
101–106	6	4	10	20	4	4	12	20
Above 106	1	2	9	12	1	4	2	7
Total	111	39	66	216	118	40	115	273
Mean	89±.7	92±1.1	97±1.0	92±.5	89±.6	94±1.3	92±.7	91±.4
Standard deviation	7±.5	7± .8	8± .7	8±.4	6±.4	8± .9	7±.5	7±.3
Mean corpuscular hemoglobin								
micro-micrograms								
Below 24	—	—	—	—	1	—	1	2
24–26	6	—	—	6	15	—	5	20
27–29	48	9	6	63	38	9	20	67
30–32	39	20	31	90	54	20	59	133
33–35	11	7	14	32	9	7	23	39
36–38	4	3	11	18	1	4	5	10
Above 38	1	—	2	3	1	—	—	1
Total	109	39	64	212	119	40	113	272
Mean	29.9±.26	31.2±.45	32.7±.47	31.0±.21	29.6±.23	31.3±.39	30.9±.25	30.4±.16
Standard deviation	2.8±.19	2.8±.32	3.8±.33	3.0±.15	2.6±.17	2.5±.28	2.7±.18	2.6±.11
Mean corpuscular hemoglobin concentration								
per cent								
Below 30	1	—	2	3	2	1	2	5
30–31	5	2	3	10	9	3	5	17
32–33	50	14	24	88	58	14	52	124
34–35	41	19	32	92	43	22	45	110
36–37	9	4	3	16	5	—	7	12
38–39	3	—	1	4	—	—	—	—
Above 39	1	—	1	2	—	—	—	—
Total	110	39	66	215	117	40	111	268
Mean	33.7±.17	33.8±.21	33.8±.23	33.7±.12	33.2±.14	33.4±.22	33.4±.14	33.3±.09
Standard deviation	1.8±.12	1.3±.15	1.8±.16	1.8±.08	1.5±.10	1.4±.16	1.5±.10	1.5±.06

that, whereas only 3% of males and 4% of females had values for mean corpuscular volume of less than 80 cubic micra, 32% of the males and 27% of females had values above 94 cubic micra. Corresponding percentages of abnormal values (Wintrobe, '29) for mean corpuscular hemoglobin are 3% of males and 8% of females with values below 27 micromicrograms, but 25% of males and 18% of females with values above 32 micromicrograms. Most of the low values for these determinations occurred among the children examined. Most of the values for mean corpuscular hemoglobin concentration fell between 32 and 37%.

TABLE 13

Serum proteins.

TOTAL PROTEIN	MALES	FEMALES	TOTAL	ALBUMIN	MALES	FEMALES	TOTAL
gm./100 cc. serum				*gm./100 cc. serum*			
Below 5.50	1	2	3	Below 3.50	2	1	3
5.50–6.00	11	1	12	3.50–4.00	20	11	31
6.00–6.50	43	43	86	4.00–4.50	45	64	109
6.50–7.00	54	73	127	4.50–5.00	75	100	175
7.00–7.50	66	95	161	5.00–5.50	41	60	101
7.50–8.00	22	33	55	Above 5.50	13	28	41
Above 8.00	6	20	26				
Total	203	267	470	Total	196	264	460
Mean	6.90±.04	7.07±.04	7.00±.03	Mean	4.69±.04	4.81±.03	4.76±.03
Standard deviation	.60±.03	.60±.03	.60±.02	Standard deviation	.55±.03	.54±.02	.55±.02

The chief hematologic abnormality is a macrocytic hyperchromic anemia. This is definitely not an iron deficiency manifestation. The association of such macrocytic anemias with diets inadequate in protein, especially animal protein, has been noted (Bethell, '36).

The levels of serum albumin are probably the most significant values presented in table 13. Eleven per cent of the males and 5% of the females had values for serum albumin below 4 gm. per 100 cc. Of the thirty-four persons with serum albumin

below this level, only five had a total protein content of less than 6 gm. per 100 cc. of serum, indicating the necessity of determining the serum protein fractions in testing for hypoproteinemia. The values for serum globulin are not presented in detail; the mean was 2.28 ± .03 gm. per 100 cc., standard deviation .67 ± .02, with no significant age or sex differences.

TABLE 14

Serum vitamin A.

INTERNATIONAL UNITS PER 100 CC. SERUM	MALES				FEMALES			
	0–5 years	6–12 years	Over 12 years	Total	0–5 years	6–12 years	Over 12 years	Total
Below 35	4	9	4	17	4	8	5	17
35– 54	11	13	9	33	9	21	13	43
55– 74	4	12	17	33	7	17	48	72
75– 94	2	9	16	27	3	7	22	32
95–114	1	5	17	23	2	5	21	28
115–134	—	2	7	9	1	3	4	8
Above 134	1	2	6	9	—	—	4	4
Total	23	52	76	151	26	61	117	204
Mean	57 ±6.2	67 ±4.5	87 ±4.1	75 ±2.9	58 ±5.2	62 ±3.4	77 ±2.5	71 ±2.0
Standard deviation	30 ±4.4	32 ±3.2	36 ±2.9	35 ±2.0	27 ±3.7	26 ±2.4	28 ±1.8	28 ±1.4
Median	51	68	83	71	56	59	71	68

Generally accepted standards for a satisfactory level of vitamin A in the serum are at present not definitely established. Most persons eating a well-balanced diet have values in the neighborhood of 100 I.U. per 100 cc. (Kimble, '39). In the present study (table 14) about one-third of the subjects had values below 55 I.U. per 100 cc. The values tend to be lower in children, and there is a probably significant sex difference among the adults, the values for males tending to be higher. The serum carotene levels show a wide range (table 15); no consistent relationship between the carotene and vitamin A levels in the serum of the same individual was noted. Both values tend to be low in the youngest age group.

TABLE 15

Serum carotene.

CONCENTRATION IN MICROGRAMS PER 100 CC. SERUM	0–5 YEARS	6–12 YEARS	13–70 YEARS	TOTAL ALL AGES
Below 50	9	7	5	21
50–100	16	18	23	57
100–150	13	29	45	87
150–200	4	28	45	77
200–250	4	18	35	57
250–300	2	10	21	33
300–350	—	4	12	16
350–400	—	2	5	7
Above 400	1	4	5	10
Total	49	120	196	365
Mean	116 ± 12	174 ± 8	191 ± 6	175 ± 5
Standard deviation	84 ± 8	93 ± 6	87 ± 4	92 ± 3
Median	98	164	182	164

TABLE 16

Serum ascorbic acid.

MG./100 CC. SERUM	ALL SUBJECTS
0.1–0.3	1
0.3–0.5	9
0.5–0.7	60
0.7–0.9	140
0.9–1.1	107
1.1–1.3	74
1.3–1.5	33
1.5–1.7	6
Total	430
Mean	0.94 ± 0.12
Standard deviation	.24 ± .008

The ascorbic acid content of the sera (table 16) indicates that nearly all the subjects were receiving an adequate intake of this vitamin.

DISCUSSION

The clinical and laboratory findings agree fairly well with what might be anticipated from the dietary records. It should be appreciated that the techniques used or available are far from equal in sensitivity in detecting early states of specific deficiency. For example, it is possible to detect vitamin C subsaturation long before clinical scurvy is imminent, but a prolonged and severe restriction of dietary protein is apparently required before the level of serum proteins will fall. The absence of reliable clinical or laboratory criteria of early nicotinic acid deficiency is keenly felt in a study of this sort. These factors, in addition to the protective effect of the low caloric content of the diet, undoubtedly affect the apparent incidence of specific qualitative deficiencies.

These observations, obviously of a localized and seasonal nature, should not be regarded as characteristic of the entire country. Observations by one of us (J.H.J.) during the fall of 1940 indicated that the food supply and state of nutrition varied a great deal from one section of the country to another. The section of nutrition in Madrid is continuing to function in collaboration with the public health authorities, and it is intended to conduct additional surveys during the coming year, as well as evaluate more adequately some of the clinical findings in the original survey by observing response to specific therapy.

SUMMARY

A study of the nutritional status of 561 persons of a low economic level was carried out in an industrial suburb of Madrid during the summer of 1941 by the combined methods of family food consumption records, individual clinical examination, and laboratory determinations.

The dietary records indicated an average intake of calories and calcium far below maintenance levels, and a protein intake of questionable adequacy. The levels of iron, phosphorus, thiamine, and ascorbic acid appeared generally adequate for maintenance; those for vitamin A and riboflavin at, or below, the border line.

Clinical examination revealed almost universal evidence of quantitative (caloric) underfeeding. Classical qualitative deficiency disease was rare: two cases of nutritional edema, one of pellagra (chronic), three of rickets. Search for early manifestations of such qualitative deficiencies disclosed a significant number of persons with skin lesions attributable to vitamin A deficiency, and a fairly high incidence, especially among the females, of capillary invasion of the cornea which is probably due to ariboflavinosis. In addition, many subjects had signs and symptoms of a mild neural or neuromuscular disturbance of undetermined origin.

Laboratory studies showed that about one-third of the subjects had a mild macrocytic hyperchromic anemia. The incidence of hypoproteinemia was 11% among males and 5% among females. Serum vitamin A levels suggest that at least one-third of the subjects may have been inadequately fed in this respect at the time of the survey. Serum ascorbic acid values indicate that nearly all had adequate body stores of this vitamin.

LITERATURE CITED

BENEDICT, F. G., AND COLLAB. 1919 Human vitality and efficiency under prolonged restricted diet. Carnegie Institution Publ. No. 280, Washington.

BETHELL, F. H. 1936 The blood changes in normal pregnancy and their relation to the iron and protein supplied by the diet. J. Am. Med. Assoc., vol. 107, p. 564.

BIGWOOD, E.-J. 1939 Guiding principles of studies on the nutrition of populations. League of Nations Technical Commission on Nutrition, Geneva.

BODANSKY, A. 1933 Phosphatase studies; determination of serum phosphatase. J. Biol. Chem., vol. 101, p. 93.

BOOHER, L. E., AND E. R. HARTZLER 1939 The vitamin B_1 content of foods in terms of crystalline thiamin. U. S. Dept. of Agriculture, Technical Bulletin No. 707, Washington.

DROESE, W., AND H. BRAMSELL 1941 Vitamin-Tabellen der Gebrauchlichstem Nahrungsmittel. J. A. Barth, Leipzig.

EVELYN, K. A. 1936 A stabilized photoelectric colorimeter with light filters. J. Biol. Chem., vol. 115, p. 63.

FARMER, C. J., AND A. F. ABT 1935 Ascorbic acid content of blood. Proc. Soc. Exp. Biol. and Med., vol. 32, p. 1625.

FISKE, C. H., AND Y. SUBBAROW 1925 Colorimetric determination of phosphorus. J. Biol. Chem., vol. 66, p. 375.

FIXSEN, M. A. B., AND M. H. ROSCOE 1940 Tables of the vitamin content of human and animal foods. II. Nutrition Abstracts and Reviews, vol. 9, p. 795.

GRANDE (COVIAN), F. 1939 La alimentación en Madrid durante la guerra. Publicaciones de la Revista de Sanidad e Higiene Pública, December.

GRANDE (COVIAN), F., AND F. JIMÉNEZ GARCIA 1940 Sobre los trastornos carenciales observados en Madrid durante la guerra. I. Los cuadros clínicos presentados con más frecuencia y su clasificación. II. La presentación en el tiempo de los distintos cuadros carenciales. Revista Clínica Española, vol. 1.

GRANDE, F., AND M. PERAITA 1941 Vitaminas y sistema nervioso. Revista Clínica Española, vol. 2, p. 209.

KIMBLE, M. S. 1939 The photocolorimetric determination of vitamin A and carotene in human plasma. J. Lab. and Clin. Med., vol. 24, p. 1055.

MAY, C. D., K. D. BLACKFAN, J. F. MCCREARY AND F. H. ALLEN 1940 Clinical studies of vitamin A in infants and children. Am. J. Dis. Children, vol. 59, p. 1167.

PUYAL, J., AND SRTA. TORRES 1932 Anales de la Casa de Salud, Valdecilla, Inst'o Medico de Postgraduados, Santander, pp. 191, 197, 213, 278.

RANDOIN, G. L. 1939 Problèmes Actuelles d'Alimentation Herrman, Paris.

ROBINSON, H. W., AND C. G. HOGDEN 1940 Biuret reaction in determination of serum proteins; study of conditions necessary for production of stable color which bears quantitative relationship to protein concentration. J. Biol. Chem., vol. 135, p. 707.

ROBINSON, W. D., J. H. JANNEY AND F. GRANDE COVIAN 1942 Studies of the physical characteristics of selected children in Madrid, Spain, in 1941. J. Pediatrics, vol. 20, p. 723.

SCHALL, H. 1941 Nahrungsmittel-tabelle zur Aufstellung und Berechnung von Diätverordnungen für Krankenhaus Sanat. und Praxis. J. A. Barth, Leipzig.

SHERMAN, H. C. 1937 Chemistry of food and nutrition. 5th ed. Macmillan, New York.

SHERMAN, H. C., AND C. S. LANGFORD 1940 Essentials of nutrition. Macmillan, New York, p. 77.

SYDENSTRICKER, V. P., W. H. SEBRELL, H. M. CLECKLEY AND H. D. KRUSE 1940 The ocular manifestations of ariboflavinosis. J. Am. Med. Assoc., vol. 114, p. 2437.

VAZQUEZ Y SANCHEZ, J. 1932 Composición química de los alimentos españoles. Gaceta Médica Española, Madrid, vol. 6, p. 62.

WILLIAMS, R. R., AND T. D. SPIES 1938 Vitamin B_1 (thiamin) and its use in medicine. Macmillan, New York.

WINTROBE, M. M. 1929 Volume and hemoglobin content of red blood corpuscle: simple method of calculation, normal findings, and value of such calculations in the anemias. Am. J. Med. Sciences, vol. 177, p. 513.

YOUMANS, J. B. 1941 Observations on nutrition in France. Trans. and Studies College of Physicians of Philadelphia, vol. 9, p. 144.

THE EFFECTS OF A DIET DEFICIENT IN PART OF THE VITAMIN B COMPLEX UPON MEN DOING MANUAL LABOR

R. E. JOHNSON, R. C. DARLING, W. H. FORBES, L. BROUHA, E. EGAÑA AND A. GRAYBIEL

The Fatigue Laboratory, Harvard University and The Thorndike Memorial Laboratory, Boston City Hospital, Boston, Massachusetts

ONE FIGURE

(Received for publication July 1, 1942)

There is urgent need for exact knowledge of the dietary needs of persons engaged in hard physical work. Surveys have shown that many manual laborers are living on diets that may be deficient in one or more essentials. Even more important at present, troops living on emergency rations, and sailors separated from bases, run the danger of having to work hard on diets that may be inadequate.

Optimal nutrition is necessary for securing the highest degree of physical fitness. Merely preventing deficiencies will not suffice. Most of the available data is concerned chiefly with either the appearance of clinical evidence of deficiency after a prolonged poor diet, or the earliest manifestations of deficiency in sedentary subjects or hospital patients. Very little is known about what happens when subjects, previously on a good diet, do heavy labor on a deficient diet. Specific practical questions are: (1) How soon does physical deterioration begin? (2) How much deterioration will occur in 1 week, the maximal period in which military emergency rations are expected to be used? (3) What are the conditions of recovery from such deficiency?

In the present experiments, ten men were subjected to heavy labor on a diet deficient in certain members of the vitamin B complex, but adequate in all other respects. Laboratory and field tests were performed to detect physical deterioration. Observations were made on the protective action of daily doses of 2 mg. of thiamine hydrochloride, and on the curative action of whole dried brewers' yeast.

EXPERIMENTAL PROCEDURES

The experiments were planned as follows:

Time table. January 7–15, preliminary measurements made, and normal diet used. January 15–22, manual labor performed while eating a deficient diet. January 23–27, manual labor performed; same basal diet as before but with added daily dose of yeast. The manual labor consisted in chopping, sawing and splitting oak logs; in building breakwaters with large stones; and in walking. The shortest hike was 7½ miles, the longest, 20. The daily requirement was between 4000 and 5000 calories.

Subjects. The subjects were ten healthy men, ranging from 23 to 40 years of age. It is important to note that the initial state of physical training was widely different from subject to subject, and also their relative physical endowments.

Diet. The diet is described in table 1. From previous experience with sedentary subjects it was known to be adequate (a) for maintenance of weight for 6 weeks; (b) in protein with respect to quantity (65 to 80 gm. per day) and in quality (cheese and egg white being a source of first-class protein); and (c) in vitamins A, C, and D. Data on the various members of the B complex in foods are incomplete, and the maintenance doses are not exactly known. However, by present standards (National Research Council,'41) the diet was certainly deficient in thiamine, probably deficient in nicotinic acid, pyridoxine and pantothenic acid, and probably adequate in riboflavin.

All of the subjects made their selections from the same group of foods, and the caloric intake was not restricted.

Capsules containing 2 mg. of thiamine hydrochloride were administered to five subjects every day at lunch, and at the same time the five others received placebos. The subjects did not know whether they received thiamine or placebos.

After 1 week of the deficient diet, the subjects began to take, instead of capsules, 18 gm. of whole dried brewers' yeast.[1] They ate the same deficient diet and continued to perform manual labor as before.

TABLE 1

Diet

Foods allowed in unlimited amounts at any meal
Cheese, rice, macaroni, spaghetti, soda crackers, butter, puffed rice (unfortified), honey, salad oil, shortening (lard or vegetable), sugar, coffee, tea, heavy cream, salt, vinegar, egg white (no yolk), baking powder biscuits (prepared from thiamine-free flour, no milk or yeast), tapioca (lemon flavor), hard candy, special cookies (made from thiamine-free flour, brown sugar, cream and water), and gelatine.
Foods allowed in small servings and with special restrictions
Either grape juice (small glass) or cranberry juice (small glass) once a day. Onions in small amounts were used at any time for flavoring.
Of the following, two and only two, were served each day: Beets (canned), spinach (boiled), lettuce (fresh), strawberries (frozen or canned), raspberries (frozen or canned), apple (one only, small), applesauce, banana (fresh), canned peaches.
Foods not specifically mentioned above were forbidden.

The yeast contained the following vitamins, in micrograms per gram, the methods used being given in parentheses: thiamine (Schultz, Atkin and Frey, '42) 20–30; riboflavin (Kemmerer, '40) 70; nicotinic acid (Melnick and Field, '40) 600; pyridoxine (Williams, Eakin and McMahon, '41) 85; pantothenic acid (Pennington et al., '41) 200. The total protein content was 45%.

It will be seen that the thiamine content of this yeast was relatively low, so that the subjects who had previously received 2 mg. of thiamine hydrochloride per day now received only ½ mg. per day.

[1] This yeast (type 2019) was donated by Standard Brands, Inc.

For purposes of clarity the subjects who received placebos will be called the "deficient" subjects, and those who received, during the first half of the experiment, 2 mg. of thiamine hydrochloride per day will be called the "thiamine" subjects. The first half of the experiment will be called the "deficient" period, since even the "thiamine" subjects failed to receive certain other needed vitamins of the B complex; the second half will be called the "yeast" period, since all subjects were then taking yeast every day.

Field measurements of physical fitness. Each day, and sometimes morning and evening, every subject's physical condition was tested quantitatively by a test devised in the Harvard Fatigue Laboratory for use in the field. It consists of pulling a stoneboat loaded so that the pull is ⅓ the subject's body weight, over a flat course 300 yards in length, at a rate of 1 yard per second. A subject may be forced to stop exhausted before 300 yards. The score is calculated from the formula:

$$\text{Fitness index} = \frac{\text{Duration of standard exhausting exercise} \times 100}{2 \times \text{sum of pulses taken from 1 to 1½, 2 to 2½, 4 to 4½ minutes of recovery}}$$

(Johnson, Brouha and Darling, '42).

A good score is above 80, a poor one below 40. This test is useful both in evaluating the physical fitness of subjects for exhausting exercise, and in following the state of training of any individual subject. Each evening private interviews were held with each subject to review the symptoms of the day.

Laboratory measurements of physical fitness. More extensive measurements of physical fitness were made on each subject on three occasions: (a) Before starting the deficient diet and the heavy work; (b) 1 week after starting the deficient diet and manual labor; (c) 5 days after beginning to take yeast along with the deficient diet and continued manual labor. The tests were carried out as follows: The subject ran on a motor-driven treadmill for 15 minutes on the level at a speed of 5.8 m.p.h., 7.0 m.p.h., or 8.6 m.p.h., depending on his physical condition. During this run the pulse rate was re-

corded continuously by means of a cardiotachometer, and ventilation, oxygen consumption, and carbon dioxide output were measured. After resting 6 minutes the subject ran at the same speed as before but at a grade of 8.6%, until he could run no longer. The heart rate was recorded continuously during the run and for 6 minutes afterwards.

After resting ½ hour, the subject again ran to exhaustion at the same grade and speed. His pulse was recorded during recovery at intervals for 6 minutes.

Other observations. Before the subjects left for the island where the experiments took place, 24-hour samples of urine were collected for estimation of thiamine; others were collected after the subjects had been on the diet 6 and 7 days. Electrocardiograms were taken and heart size estimated by x-ray in the normal, in the deficient, and, in some cases, in the yeast periods.

RESULTS

Caloric value of the diet. The requirement of the subjects was calculated to be from 4000 to 5000 calories a day. The caloric value of the diet was adequate since all subjects went through both the deficient and the yeast periods without significant change in weight. The maximal change of any subject's weight was 1.5 kg., and the average net weight change was —0.3 kg.

Urinary findings. Thiamine in the urine was estimated by the method of Egaña and Meiklejohn ('41). At the end of the deficient period, the five "deficient subjects" were excreting thiamine in amounts well within the range usually taken as indicative of deficiency (Robinson, Melnick and Field, '40); the average excretion was 29 μg. of thiamine in 24 hours. The five "thiamine subjects" excreted amounts of thiamine well up in the normal range; the average was 200 μg. in 24 hours. The urinary findings prove conclusively that the "deficient subjects" depleted their stores of thiamine significantly within 7 days.

Symptoms. The "deficient subjects" all developed within 6 days a majority of the following symptoms, sometimes

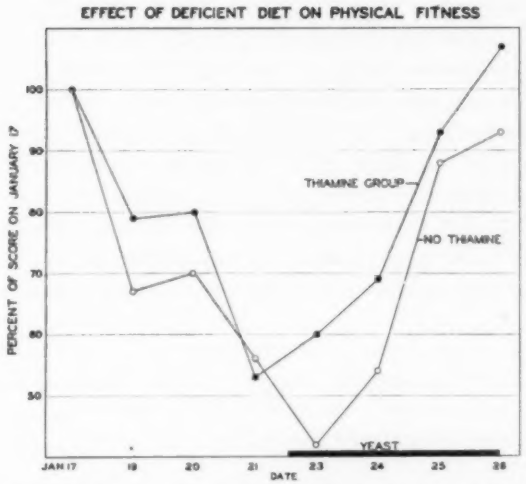
acutely: (a) A general feeling of lassitude, inefficiency and depression. (b) Easy fatigue, especially in climbing stairs and hills. (c) Muscle and joint pains different from the stiffness following unaccustomed exercise: instead of disappearing after a few days of exercise, they grew steadily worse. The pains came abruptly after motion was started, but did not diminish during the day's work and stopped a few hours after work was over. (d) Poor appetite and even antipathy to food, even though the subjects forced themselves to eat enough to maintain weight. (e) Irregular bowel habits taking the form of severe constipation or scanty, hard stools. (f) "Slowness" was a vague but prominent symptom, the subjects being unable to accomplish fast motion when they wished. (g) Additional psychic changes in the form of quarrelsomeness and irritability appeared in two subjects.

In contrast to the "deficient subjects," the "thiamine subjects" had few symptoms, and these were mild. However, one complaint common among them was easy fatigue.

Following the administration of yeast, there was a lag of 48 hours before the subjects began to feel better. Then the improvement in feeling occurred very suddenly, and could be timed almost to the hour. By the end of 5 days no subject had any complaints although the same diet (with the addition of yeast only) was still being eaten.

Results of measurements of physical fitness in the field. The results of the daily measurements of fitness are shown in figure 1. It will be seen that both groups of subjects showed marked deterioration in physical state within 6 days, but those receiving thiamine showed this in somewhat less degree. After yeast was administered, all subjects recovered, the thiamine subjects quicker and more completely than the others.

Results of measurements of physical fitness on the treadmill. In general, for moderate exercise (i.e., running on the level) the "deficient subjects" showed, in the deficient period, abnormally high pulmonary ventilation and moderately increased oxygen consumption, whereas the "thiamine subjects" altered only slightly in these respects. Ventilation was increased especially in those subjects who had complained most



Deterioration in physical fitness for hard work following subsistence on a diet deficient in the vitamin B complex, and improvement following daily addition of whole brewers' yeast to the deficient diet, starting on January 22. Open circles: Average for "deficient subjects." Dots: Average for "thiamine subjects."

of easy fatigue. For instance, the ventilation of the deficient subject *We* was 890, 1062 and 869 cc. per kilo per minute in the control, deficient and yeast periods, respectively, and the corresponding oxygen consumption was 46.5, 48.3 and 47.5 cc. per kilo per minute. This phenomenon is comparable to what has been seen in sedentary subjects submitted to a vitamin B deficient diet for at least 4 weeks.[2] These findings indicate an impaired mechanical efficiency [3] for moderate work.

[2] Recent observations from these Laboratories (Egaña et. al., '42).

[3] The term "mechanical efficiency" is used in this paper in its strict sense, i.e., the ratio of effective external work performed to the total caloric output during work that is maintained in a steady state. The tests of physical fitness used on our subjects are designed to test endurance for long sustained exertion, such as walking 40 miles (Johnson, Brouha and Darling, '42). Recuperation is measured by repetition of the standard exhausting exercise. The performance of the second bout of exhausting exercise is an indication of the effectiveness of the recovery processes which enable a man to carry out repeated exhausting tasks. This type of recovery, when effective, enables some athletes to run both the mile race and the 2-mile race on a single afternoon. It is of the greatest importance to manual laborers, if they are to work effectively.

Two conclusions can be drawn from the performance of exhausting exercise, i.e., running uphill (table 2): (1) Both groups of subjects exhibited marked deterioration during the deficient period, especially in the capacity to perform repeated exhausting exercise. This is directly opposite to what was seen in two of the same group of men in a later experiment, when they did daily manual labor while subsisting on a normal diet. (2) Both groups of subjects improved strikingly during the yeast period. In fact, as a result of training, seven became fitter than they had been when eating a normal diet.

TABLE 2

Response of two individual subjects to repeated exhausting exercise at 7.0 m.p.h. and 8.6% grade.

Results are expressed as fitness indices (Johnson, Brouha and Darling, 1942).

	DEFICIENT SUBJECT HOL		THIAMINE SUBJECT JOH	
	1st run score	2nd run score (½ hr. later)	1st run score	2nd run score (½ hr. later)
A. Period of normal diet	133	141	83	86
B. "Deficient" period	122	105	63	67
C. "Yeast" period	138	107	85	95

Cardiological observations. No significant changes were observed in the electrocardiogram of most subjects. In two instances slight lowering of the T waves was observed and may have been due to the vitamin deficiency.[4] Because of the small magnitude of the changes, the electrocardiogram is of little help in detecting early stages of this type of vitamin deficiency. However, a significant increase in the amplitude of T following the administration of vitamin B complex would strongly suggest that a state of deficiency existed previously.

Teleroentgenograms of the chest taken before and at the end of the deficient period showed no significant difference in heart size.

[4] The "deficient subject" WY developed cheilosis of marked degree, and a sore tongue, at which time the electrocardiogram showed nodal rhythm with significantly lower T waves in Leads I, II, and CF4.

DISCUSSION

The practical conclusion of these experiments is that any person engaging in daily hard physical work needs imperatively an adequate intake of the vitamin B complex every day if he is to remain fit. Brewers' yeast was a complete and adequate supplement to the deficient diet used. It abolished in every subject the symptoms and signs of deficiency. In the diets of workers, natural food products can supply the necessary daily vitamin intake in a palatable form and without risk of deficiency. If extra vitamins are ever indicated for any ration, addition of brewers' yeast at present seems a sure way of avoiding deficiency in any single component of the B complex.

Certain observations deserve special emphasis: (1) Our subjects developed symptoms and definite signs of deterioration far more quickly and more severely than sedentary subjects on a similar diet (e.g., the subjects of Williams, Mason, Wilder and Smith, '40). (2) Recovery after deficiency was a relatively slow process, and three of the "deficient subjects" were not yet completely normal after taking yeast for 5 days. (3) The abnormally increased ventilation and oxygen consumption during moderate work which was especially marked in three deficient subjects would be a great disadvantage where air and oxygen are at a premium, e.g., at high altitudes and in diving operations. (4) Impairment of the ability to carry out repeated exhausting tasks is a great disadvantage to anyone engaged in sustained physical exertion.

It is worth emphasizing that marked physical deterioration occurred in spite of the ingestion of 2 mg. of thiamine hydrochloride per day which clearly prevented the thiamine group from experiencing the muscle pains and other symptoms reported by the "deficient subjects." When yeast, containing only $\frac{1}{2}$ mg. of thiamine per day, was administered, physical improvement occurred. It seems possible from these results to distinguish some of the characteristics of both early thiamine deficiency and early deficiency with respect to other parts of the vitamin B complex. The "deficient subjects" had

marked symptoms, the "thiamine subjects," few. Thiamine deficiency would therefore seem to cause symptoms. Both groups of subjects developed marked signs of deterioration, the "deficient subjects" more than the others. This moderate extra deterioration would seem to be caused by thiamine deficiency in addition to the other deficiencies. Presumably, the thiamine hydrochloride did not prevent the deterioration which was cured by the yeast. This conclusion is strengthened by the fact that both groups recovered their physical fitness while taking yeast which contained thiamine in doses only one-quarter as large as the "thiamine subjects" had previously received.

For these reasons, in working men, we consider early thiamine deficiency to be characterized by marked symptoms and moderate physical deterioration, and early deficiency in other members of the vitamin B complex to be characterized by few symptoms but marked physical deterioration.

SUMMARY

Ten men were subjected to hard daily physical work on a diet deficient in parts of the B complex, notably in thiamine, and adequate in all other respects. During the first week of the experiment five subjects received 2 mg. of thiamine hydrochloride per day, the other five, placebos. During the last week of the experiment all subjects received daily doses of 18 gm. of brewers' yeast containing ½ mg. of thiamine.

The main results were as follows: (a) At the end of 1 week all subjects complained of easy fatigue and their physical fitness had deteriorated markedly; this was greater in the subjects without thiamine. (b) A majority of subjects without thiamine exhibited symptoms, sometimes acute, of muscle and joint pains, lack of well-being, poor appetite and constipation. These symptoms were mild or absent in the subjects receiving thiamine. (c) During the "yeast period" all symptoms disappeared and the usual level of fitness was regained more rapidly and more completely by the subjects who had received thiamine during the first week. (d) Changes in the electro-

cardiograms of certain subjects in both groups that were noticed at the end of the deficient period had disappeared by the end of the yeast period. (e) At the end of the first week, the daily urinary excretion of thiamine by the subjects who had not received this vitamin was low and well within the range characteristic of deficiency.

The following conclusions are drawn: (a) When men are doing hard physical work even for a few days, there is an imperative need for an adequate daily intake of the vitamin B complex if physical fitness is to be maintained. (b) Of the vitamin B complex thiamine alone will not maintain the physical fitness of laborers in single daily doses of 2 mg. (c) Whole dried brewers' yeast, on the other hand, is a complete and adequate supplement for a diet grossly deficient in the vitamin B complex. (d) When addition of vitamin B complex to a ration is indicated, a natural product such as yeast would seem to be a sure source of all the necessary components.

ACKNOWLEDGMENTS

We wish to thank the Trustees of Naushon Island for their kindness in making available to us the facilities on the island; Mr. Frank Consolazio and Mr. William Holmes, for their technical help and for acting as subjects; Drs. Jeffries Wyman, Jr., Henry Marcy, Julio Barman and Manuel Moreira, for acting as subjects; and Mrs. Barman and Mrs. Consolazio, for taking charge of the food supply.

LITERATURE CITED

EGAÑA, E., R. E. JOHNSON, R. BLOOMFIELD, L. BROUHA, A. P. MEIKLEJOHN, J. WHITTENBERGER, R. C. DARLING, C. HEATH, A. GRAYBIEL AND F. CONSOLAZIO 1942 The effects of a diet deficient in the vitamin B complex on sedentary men. Am. J. Physiol., vol. 137, p. 731.

EGAÑA, E., AND A. P. MEIKLEJOHN 1941 The estimation of thiamine in urine. J. Biol. Chem., vol. 141, p. 859.

JOHNSON, R. E., L. BROUHA AND R. C. DARLING 1942 A test of physical fitness for strenuous exertion. Revue Canadienne de Biologie, vol. 1, p. 491.

KEMMERER, A. R. 1940 Report on riboflavin (assay). J. Assoc. Off. Agric. Chem., vol. 23, p. 346.

MELNICK, D., AND H. FIELD, JR. 1940 Chemical determination of nicotinic acid: Inhibitory effect of cyanogen bromide upon the aniline side reactions. J. Biol. Chem., vol. 135, p. 53.

NATIONAL RESEARCH COUNCIL, COMMITTEE ON FOOD AND NUTRITION 1941 Recommended Dietary Allowance, Washington, D. C.

PENNINGTON, D. E., E. E. SNELL, H. L. MITCHELL, J. R. MCMAHON AND R. J. WILLIAMS 1941 Assay method for pantothenic acid. Univ. Texas Publication No. 4137, p. 14. Univ. of Texas, Austin.

ROBINSON, W. D., D. MELNICK AND H. FIELD, JR. 1940 Urinary excretion of thiamine in clinical cases and the value of such analyses in the diagnosis of thiamine deficiency. J. Clin. Invest., vol. 19, p. 399.

SCHULTZ, A. S., L. ATKIN AND C. N. FREY 1942 Determination of vitamin B_1 by yeast fermentation method. Improvements related to use of sulfite cleavage and a new fermentometer. Ind. & Eng. Chem. (Anal. Ed.), vol. 14, p. 35.

WILLIAMS, R. J., R. E. EAKIN AND J. R. MCMAHON 1941 Assay method for pyridoxin. Univ. Texas Publication No. 4137, p. 24. Univ. of Texas, Austin.

WILLIAMS, R. D., H. L. MASON, R. M. WILDER AND B. F. SMITH 1940 Observations on induced thiamine (vitamin B_1) deficiency in man. Arch. Int. Med., vol. 66, p. 785.

RELATIONSHIP BETWEEN VITAMIN A AND IODINE METABOLISM IN THE RAT [1, 2]

ROE E. REMINGTON, PHILIP L. HARRIS [3] AND CECIL L. SMITH
Department of Nutrition, Medical College of the State of South Carolina, Charleston

(Received for publication July 13, 1942)

In 1936 Remington and Levine showed that in rats maintained on an iodine deficient regime, certain quantitative relationships exist in the weight, iodine content, and dry matter content of the thyroid glands, and between each of these and the iodine supply. These criteria, together with histological examinations, have since been used in studying the effect upon the gland of superposing dietary or other deficiencies or abnormalities upon the iodine deficiency (Remington, J. W., '37; Remington, R. E., '38 a, '38 b; Harris and Remington, '39) with the hope of adding to our knowledge of the specific relationships of the thyroid in general metabolism, as well as to reveal contributory or secondary causes of endemic goiter if such exist.

The primary object in undertaking the experiments to be reported herein was to determine whether lack of vitamin A could enhance the severity of goiter due to an iodine deficiency. However, considerable work has been done and evidence accumulated bearing on other phases of the thyroid-vitamin A relationship, and our experimental approach has therefore been extended to include such questions as the effects of goiter, hypo- and hyperthyroidism on carotene utilization and vitamin A metabolism and requirement.

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] Presented before the American Society of Biological Chemists, Toronto, April 1939.

[3] Now associated with Distillation Products, Incorporated, Rochester, N. Y.

According to McCarrison ('30) the thyroid is unable to deal with iodine in a normal manner in the absence of a sufficiency of vitamin A, and goiter results. Supporting evidence is that of Mitzkewitsch ('34) who reported that in rats which died of avitaminosis A the thyroids were enlarged but their microscopic appearance indicated hypofunction, while de Ruyter ('34) found atrophy of the thyroid in severe vitamin A deficiency. In neither case is there reported any control over the iodine intake of the animals, but similar results have been reported by Coplan and Sampson ('35), the iodine content of whose diets was known. On the other hand Sherwood and Luckner ('35) observed hyperplastic thyroids, deficient in colloid, in rats fed large doses of vitamin A in the form of cod liver oil, halibut liver oil, or carotene.

Another phase of the thyroid-vitamin A relationship that has received attention is the effect of vitamin A on hyperthyroidism. Abelin ('35) has shown that the toxic effects of ingested thyroid substance or injected thyroxine can, in a measure, be offset by vitamin A. According to Logaras and Drummond ('38) such an effect can be observed with massive doses of vitamin A, but is negligible or absent when the vitamin A intake is sufficient only to supply the needs of the animal. Clinically vitamin A has been found of value in relieving symptoms of toxic goiter (Wendt, '35 a; Dietrich, '36), and Abelin ('36) recommends doses of 150,000 rat units per day in the treatment of hyperthyroidism.

There is also some evidence bearing on effects of thyroid activity on vitamin A and carotene metabolism. Wendt ('35 b) reported impaired ability to convert carotene into vitamin A in myxedema and cretinism, and Fasold and Heidemann ('33) found that after thyroidectomy goats secreted milk containing carotene but no vitamin A, whereas normally the reverse was true. This they explained by saying that the lowered general metabolism after thyroid removal limits the ability to transform carotene to vitamin A. The time required to deplete rats of vitamin A is markedly prolonged by thyroidectomy, but shortened when intact animals are injected with thyroxine

(Greaves and Schmidt, '36). The administration of carotene protects against loss of weight in rats injected with thyroxine (Euler and Klussmann, '32); and thyroxine protects against the harmful effects of massive doses of vogan (a vitamin A concentrate) according to Fasold and Peters ('33). Clausen and McCoord ('38) found that in fevers, including artificial hyperthermia, there is a drop in both the carotene and vitamin A content of human blood. This has been confirmed by Thiele and Scherff ('39 a, b) who also reported that these values are always low in Graves' disease, but not in colloid goiter.

EXPERIMENTAL

Deficiency of both vitamin A and iodine: Effect on thyroid of feeding vitamin A and carotene with and without iodine

Inspection of a number of diets previously investigated as to goiter production and growth (Remington, R. E., '37) revealed that a diet of wheat gluten, yellow corn meal, dried brewers' yeast, calcium carbonate and salt would prove satisfactory in producing a degree of goiter which would lie on a critical part of the curve, and could also be made deficient in vitamin A by replacing the yellow corn meal with sucrose. It seemed desirable, furthermore, when using so highly purified a diet, to add a more complete salt mixture, for which purpose mixture 351 of Hubbell, Mendel and Wakeman ('37) was made up without potassium iodide. The diet selected consisted of sucrose 70 parts, wheat gluten 18, dried brewers' yeast 5, cottonseed oil (containing enough viosterol to yield 3 U.S.P. units of vitamin D per gram of diet) 5, and salt mixture 2 parts. The first experiment was designed to compare the degree of goiter existing in rats equally deprived of iodine, but receiving graded levels of vitamin A as such or as carotene. The source of vitamin A was a concentrate prepared from fish liver oil by molecular distillation,[4] and the carotene a commercial product[5] stated to contain 90% beta-

[4] Distillation Products, Inc., Rochester, N. Y.
[5] S M A Corporation, Cleveland, Ohio.

and 10% alpha-carotene. Weanling rats were placed on the diet described above and maintained without addition of any supplement until depleted of vitamin A, as evidenced by a cessation of growth for 7 days, at which time they were divided into groups of eight animals each, and fed either vitamin A

TABLE 1

Effect on the thyroid gland of feeding vitamin A and carotene, with and without iodine supplementation, to rats previously depleted as to both vitamin A and iodine.

SUPPLEMENT U.S.P. UNITS PER WEEK	WEIGHT GAIN IN 5 WEEKS	THYROID GLANDS		
		Weight per 100 gm. body wt.	Dry matter	Iodine dry basis
A. Diet deficient in both vitamin A and iodine				
	gm.	*mg.*	%	%
none	—1.5 (in 2 weeks)	23.4	22.8	.020
6 vitamin A	7.9	27.8	23.1	.012
60 vitamin A	13.8	26.2	21.8	.018
600 vitamin A	56.0	27.2	25.0	.019
6 carotene	8.8	31.6	22.8	.018
60 carotene	16.4	24.7	24.3	.025
600 carotene	62.4	29.0	21.7	.020
B. Diet deficient in vitamin A but supplying about 2 μg. per day of iodine				
none		13.9	28.5	.180
6 vitamin A		14.3	26.9	.204
60 vitamin A		13.0	27.7	.192
6 carotene		15.7	30.4	.200
60 carotene		15.2	29.0	.220
Colony ration (fully adequate)		10.0	33.3	.200

or carotene at levels of 3, 30, and 300 U.S.P. units twice weekly for 6 weeks, rats of the control group meanwhile receiving just enough vitamin A to keep them alive. The results [6] are shown in the first part of table 1. From previous experience with diets of this type, that degree of thyroid hyperplasia which any given diet is capable of producing will be attained

[6] The glands from each group were assembled in a small nickel boat and, after drying, handled essentially as described by Remington et al. ('30), except that the final estimation was made by a micro-modification of the thiosulphate titration.

in 5 weeks or less, after which the thyroid values remain rather constant. Since the average time required for depletion in vitamin A was more than 5 weeks, what was being observed here was the ability of vitamin A and carotene to render more efficient the utilization of the unavoidable minimum of iodine in the diet for the relief of a previously existing thyroid hypertrophy. It will be seen from the data that no significant differences are apparent in the constants on the thyroid glands of the different groups, i.e., vitamin A and carotene at the levels fed are not able to render more efficient the use of iodine in such minute amounts by hyperplastic glands.

It may be that in the above experiment the iodine restriction is so severe as to nullify any possible effect of the vitamin, in which case it would be more logical to perform the experiment on rats receiving nearly, but not quite, a fully protective daily dose of iodine. The experiment was repeated, with the difference that sodium iodide was added to the diet in an amount that would provide an average daily intake of 2 μg. iodine per rat, this being slightly less than the 3 μg. which we consider the minimum adequate level for normal thyroid composition and structure. Here again (table 1, part B) although the thyroid constants are quite different from those of the former experiment, and more nearly like those of normal animals (Remington, Remington, and Welch, '37), there is no definite indication that the addition of vitamin A or carotene has affected either the degree of enlargement or the utilization of iodine by the thyroid.

Deficiency of vitamin A: Effect of administering iodide and desiccated thyroid on eye symptoms and survival

Since vitamin A is apparently without effect upon the development of low iodine goiter under the conditions of the preceding experiment, a study was next undertaken to determine whether the converse is true, i.e., whether a degree of goiter, or a deficiency in the thyroid hormone itself, would have any effect on the requirement for or utilization of vitamin A. Six groups of weanling rats were placed on the same

diet as before, with various supplements as indicated, from the beginning, as shown in table 2. Animals of the control group, receiving only the basal diet, developed xerophthalmia after an average period of 51 days, and died within 6 or 7 days with typical post-mortem symptoms of avitaminosis A as well as enlarged and hyperplastic thyroids. Addition of

TABLE 2

Effect of administering iodide and desiccated thyroid gland, on the incidence of eye symptoms and the survival period of normal and thyroidectomized rats deprived of vitamin A.

NUMBER OF ANIMALS	TREATMENT	SUPPLEMENT (FED DAILY)	APPEARANCE OF EYE SYMPTOMS	SURVIVAL PERIOD	REMARKS
			(days)	*(days)*	
10	...	.....	51	58	Thyroids definitely hyperplastic
10	...	5 μg. iodine	49	60	Thyroids normal
10	...	20 mg. thyroid per 100 gm. body weight	28	35	Thyroids normal
5		20 mg. thyroid per 100 gm. body weight plus 5 μg. iodine	30	35	Thyroids normal
8	Thyroids removed	20 mg. thyroid per 100 gm. body weight	55	61	At autopsy gross and microscopic examination failed to reveal any vestiges of remaining thyroid tissue
8	Thyroids removed	.....	83	98	

5 μg. per day of iodine as sodium iodide did not affect the vitamin A depletion time or the survival time, but the thyroids were grossly and histologically normal. A third group received daily doses of commercial desiccated thyroid gland in an amount which, in animals of our stock colony, would result in slightly decreased growth. Here the onset of xerophthalmia occurred in 28 days, death following in 7 days more; this is

a marked shortening of the vitamin A depletion time which we, in the absence of more specific indications, are inclined to attribute to the heightened general metabolism due to the desiccated thyroid. The thyroid glands were normal, having been spared the effects of the iodine deficient diet by the desiccated thyroid. This picture is not affected when 5 μg. per day of iodine is given with the desiccated thyroid. Two additional groups were thyroidectomized at 28 days of age, 6 days after being placed on the experimental diet. Of these, one group received desiccated thyroid, the other no supplement. In the thyroid-fed group the vitamin A depletion time and survival period were substantially the same as those of unoperated animals without dietary supplement, but the thyroidectomized group without supplement, while not making much gain in weight after the operation, continued in apparent good condition for an average of 83 days before symptoms of vitamin A deficiency appeared; they survived for 15 days longer, dying with typical symptoms of avitaminosis A. These observations confirm the findings of Greaves and Schmidt. However, not being able to reproduce the effect of excess thyroxine with dinitrophenol, they did not believe that the change in basal metabolic rate was responsible for the change in the rate of vitamin A depletion. On the other hand, the finding of Clausen and McCoord that plasma vitamin A values are lowered in fevers and artificial fevers, as well as in toxic goiter, would imply that there is an increased need for vitamin A which parallels, at least, the general heat production. All of the thyroidectomized rats were not examined at autopsy for histological evidence of complete thyroid removal, but seven representative animals were so examined and no evidence of functional thyroid tissue found.[7] Comparing the depletion time of the thyroidectomized rats with that of intact animals, with and without iodine, reveals that a degree of iodine deficiency which brings about a decrease of 90% in the iodine storage of the thyroid, with a corresponding

[7] We are indebted to Dr. C. A. Swinyard, formerly of the Department of Anatomy of this institution, for the careful histological studies involved in our experiments.

decrease in colloid and other characteristic changes, has no effect on the vitamin A depletion time, whereas complete removal of the thyroid does. It must be that even so severe a degree of goiter as is represented in these experiments has an insignificant effect on the general metabolism as compared with complete extirpation of the gland. In confirmation of the above, one of us (R. E. R.) has determined the basal metabolic rate of a considerable number of iodine-deficient rats over a period of several years, with the conclusion that there is only a slight and erratic tendency toward a rate lower than that of normal animals.

One other postulation can be tested by this technique, i.e., that hypofunction of the thyroid decreases the ability of the organism to convert dietary carotene into vitamin A. In the first experiment we observed that gain in weight, when equal supplements of vitamin A and carotene were fed to depleted, goitrous rats, was practically the same for either form, thus indicating complete conversion. However, it is only an assumption that the goiter was severe enough to affect the functional state of the gland as related to general metabolism. Hence, a number of rats were thyroidectomized and maintained on the basal diet until weight was constant or declining for 7 days, when they were divided into groups and given, respectively, 1, 3, and 30 U.S.P. units per day of vitamin A or carotene. Rather rapid alleviation of eye symptoms occurred at the highest level of either vitamin source. When 1 unit of either source was fed the cure required 7 to 9 days; with 3 units, 4 days were sufficient for either source. Even at the low critical level of 1 unit per day there was no evidence that carotene was any less efficient than vitamin A in relieving xerophthalmia. In this experiment the animals were considered as being depleted of their vitamin A stores 7 days after the first appearance of a bloody exudate from the eyes, regardless of weight. Cure of xerophthalmia was considered to have occurred when the eyes had returned to as near normal as possible. Experience in conducting vitamin A bioassays enables one to judge this "cure" with considerable consistency.

Due to these rats having been thyroidectomized, there was little or no stimulation of growth at any level of vitamin A supplementation.

CONCLUSIONS

It may be concluded from these experiments, that in rats:

1. There is no reason to believe that vitamin A deficiency is an etiological factor in the development of simple goiter.

2. There is apparently an increased need for vitamin A in hyperfunction of the thyroid, probably corresponding to the increase in the rate of general metabolism, and a correspondingly decreased need in thyroidectomized animals; but in a degree of goiter in which the iodine storage of the thyroid is decreased by 90%, the latter effect is not demonstrated.

3. A deficiency of the thyroid hormone does not adversely affect the ability of the animal to utilize carotene in the cure of xerophthalmia, as compared with pre-formed vitamin A.

LITERATURE CITED

ABELIN, I. 1935 Ueber den Antagonismus A-Vitamin: Thyroxin. Ztschr. f. Vitaminforsch., vol. 4, p. 120.

———— 1936 Ueber die medikamentöse Anwendung des A-Vitamins bei der Hyperthyreose. Schweiz. med. Wchschr., vol. 66, p. 1106.

CLAUSEN, S. W., AND A. B. MCCOORD 1938 Carotenoids and vitamin A in the blood. J. Pediat., vol. 13, p. 635.

COPLAN, H. M., AND M. M. SAMPSON 1935 The effects of a deficiency of iodine and vitamin A on the thyroid gland of the albino rat. J. Nutrition, vol. 9, p. 469.

DIETRICH, H. E. 1936 Vitaminbehandlung dur Schilddrüsenüberfunktion mit Vogan. München. med. Wchschr., vol. 83, p. 313.

EULER, H. v., AND E. KLUSSMANN 1932 Carotin (Vitamin A) und Thyroxin. Ztschr. f. physiol. Chem., vol. 213, p. 21.

FASOLD, H., AND E. R. HEIDEMANN 1933 Ueber die Gelbfärbung der Milch thyreopriver Ziegen. Ztschr. f. d. ges. exper. Med., vol. 92, p. 53.

FASOLD, H., AND H. PETERS 1933 Ueber den Antagonismus zwischen Thyroxin und Vitamin A. Ztschr. f. d. ges. exper. Med., vol. 92, p. 57.

GREAVES, J. D., AND C. L. A. SCHMIDT 1936 Studies on the vitamin A requirement of the rat. Am. J. Physiol., vol. 116, p. 456.

HARRIS, P. L., AND R. E. REMINGTON 1939 The effect of yeast and of thiamin on the production of low iodine goiter. J. Nutrition, vol. 17, p. 31.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, p. 273.

LOGARAS, G., AND J. C. DRUMMOND 1938 Vitamin A and the Thyroid. Biochem. J., vol. 32, p. 964.

MCCARRISON, R. 1930 Further researches on lymph-adenoid goitre in rats. Indian J. Med. Research, vol. 18, p. 577.

MITZKEWITSCH, M. S. 1934 Der Zustand der endokrinen Drüsen während der A-Avitaminose bei Ratten. Arch. f. exper. Path. u. Pharmakol., vol. 174, p. 339.

REMINGTON, J. W. 1937 Low iodine goiter and the resistance of the rat to thyreotropic stimulation. Proc. Soc. Exp. Biol. Med., vol. 37, p. 175.

REMINGTON, R. E. 1937 Improved growth in rats on iodine deficient diets. J. Nutrition, vol. 13, p. 223.

———— 1938 a Does fat in the diet affect the thyroid? J. Nutrition, vol. 16, p. 417.

———— 1938 b The effect of chloride on the thyroid gland. Proc. Soc. Exp. Biol. Med., vol. 37, p. 652.

REMINGTON, R. E., AND H. LEVINE 1936 Studies on the relation of diet to goiter. III. Further observations on a goitrogenic diet. J. Nutrition, vol. 11, p. 343.

REMINGTON, R. E., J. F. MCCLENDON, HARRY VON KOLNITZ AND F. B. CULP 1930 The determination of traces of iodine. IV. Iodine in small quantities of thyroid and other tissues. J. Am. Chem. Soc., vol. 52, p. 980.

REMINGTON, R. E., J. W. REMINGTON AND S. S. WELCH 1937 The thyroid gland of the normal rat: Size, dry matter and iodine content. Anatomical Record, vol. 67, p. 367.

DE RUYTER, TH. H. 1934 Investigations into the histology of the A-avitaminosis. I. Alterations in organ and tissue structure. Acta brev. Neerland., vol. 4, p. 122.

SHERWOOD, T. G., AND W. G. LUCKNER 1935 Further studies on the effect of cod liver oil on the thyroid gland. J. Nutrition, vol. 9, p. 123.

THIELE, W., AND I. SCHERFF 1939 a Über die pathogenetische und diagnostische Bedeutung des Carotin- und Vitamin A-Spiegels im Serum. Klin. Wochenschr., vol. 18, p. 1208.

———— 1939 b Der Serum-Vitamin A-Spiegel in Fieber. Klin. Wochenschr., vol. 18, p. 1275.

WENDT, H. 1935 a Ueber die Behandlung der Basedow'schen Krankheit mit grossen Dosen Vitamin A (Vogan). Munchen. med. Wchschr., vol. 82, p. 1160.

———— 1935 b Ueber Veränderungen im Karotin-Vitamin A-Haushalt beim Myxödem und bei Kretins. Munchen. med. Wchschr., vol. 82, p. 1679.

INDEX

ABSORPTION and retention of carotene and vitamin A by hens on normal and low fat rations 199
—— intestinal, of galactose in rat, effect of vitamin B deficiency on 495
—— of dietary phosphorus by rat, influence of aluminum sulfate and aluminum hydroxide upon 111
Acid(s), amino, for chick, soybean protein as source of 385
—— ascorbic, content of ewes' blood, colostrum and milk and effect of ascorbic acid injections 121
—— —— requirements of children of early school age, study of 25
—— deficiency, pantothenic, effect of, on blood lipoids of dog 273
—— linoleic, arachidonic, and linolenic, effectiveness of, in reproduction and lactation 213
—— —— pyridoxine and pantothenic acid in rat dermatitis 225
—— nicotinic, and riboflavin, urinary excretion of, in dogs and rats, effect of level of protein intake upon 295
—— —— content of meat 153
—— —— in foods 235
—— —— pantothenic acid and pyridoxine in wheat and wheat products 167
—— pantothenic, and pyridoxine, protection afforded by, Sensory neuron degeneration in pigs. IV. 345
—— —— linoleic acid and pyridoxine in rat dermatitis 225
—— —— nicotinic acid and pyridoxine in wheat and wheat products 167
—— —— thiamine, riboflavin, and pyridoxine deficiencies, influence of, on nitrogen metabolism 405
Acrodynia, rat, body fats in 393
Action, lipotropic, of choline, relation of B-vitamins and dietary fat to 175
Albino rat, thiamine requirement of, as influenced by substitution of protein for carbohydrate in diet ... 317
Alcohol or whisky, polyneuropathy in thiamine deficient rats delayed by 73
Alcohols, mobilization by, of vitamin A from its stores in tissues 1
ALMQUIST, H. J., E. MECCHI, F. H. KRATZER AND C. R. GRAU. Soybean protein as a source of amino acids for the chick 385
Aluminum hydroxide and aluminum sulfate, influence of, upon absorption of dietary phosphorus by rat 111
Amino acids for chick, soybean protein as source of 385
Analytical values of diets, comparison of calculated with, chemical composition of twenty-two common foods and 41
Anterior pituitary extract, periodic administration of, as affecting metabolism of rats on diets of different composition 535
—— —— —— saline, effects of prolonged daily treatment of normal rats with. I. Sexual differences in appetite, growth and organ weights 469
II. Protein and energy metabolism 481
Arachidonic, linoleic, and linolenic acids, effectiveness of, in reproduction and lactation 213
ARCHER, H. E., M. KRISS AND L. VORIS. Periodic administration of anterior pituitary extract as affecting the metabolism of rats on diets of different composition ... 535
Arginine and leucine, metabolism of, with special reference to respiratory exchange and heat production 547
Ascorbic acid content of ewes' blood, colostrum and milk and effect of ascorbic acid injections 121
—— —— requirements of children of early school age, study of 25
ASHBURN, LLEWELLYN L. See Lowry, Sebrell, Daft and Ashburn 73
AXELROD, A. E. See Potter, Axelrod and Elvehjem 449

BACTERIA, yeast and rats, response of, to peroxide-treated biotin. Intestinal synthesis of biotin in rat 523
BAILEY, ERNEST A., JR. See Satterfield, Bailey, Foster and Hostetler 121
BAILEY, M. I., AND A. W THOMAS. The thiamine and riboflavin contents of citrus fruits 85
Balances, potassium, sodium and chlorine, of pre-school children receiving medium and high protein diets 437
Basal diets either low or high in phosphorus, salt mixture for use with 245
BAUM, W. S. See Clausen, Baum, McCoord, Rydeen and Breese 1
B complex factors, inhibition of symbiotic synthesis of, by sulfonamides 427
BEACH, E. F., D. M. TEAGUE, O. D. HOFFMAN, B. R. MUNKS, F. C. HUMMEL, H. H. WILLIAMS AND I. G. MACY. The sulfur metabolism of children 257
BERRYMAN, GEORGE H. See McVicar and Berryman 235
Biotin in rat, intestinal synthesis of. Response of bacteria, yeast and rats to peroxide-treated biotin ... 523
Blood, colostrum and milk, ewes', ascorbic acid content of, and effect of ascorbic acid injections .. 121
—— lipoids of dog, effect of pantothenic acid deficiency on 273

Body fats in rat acrodynia 393
BOWMAN, ROBERT S. See Kriss and Bowman 547
—— See Voris, Kriss, Marcy and Bowman 469, 481
BRAY, MERLE M. See Hawks, Bray, Wilde and Dye 283
—— See Hawks, Bray, Hartt, Whittemore and Dye 437
BREESE, B. B. See Clausen, Baum, McCoord, Rydeen and Breese ... 1
BRODY, SAMUEL. See Kibler and Brody 461
BROUHA, L. See Johnson, Darling, Forbes, Brouha, Egaña and Graybiel 585
B-vitamins and dietary fat, relation of, to lipotropic action of choline 175

CALCIUM, phosphorus and nitrogen, interrelationship of, in metabolism of pre-school children 283
—— —— —— —— metabolism of young college women 367
Calculated values of diets, comparison of analytical with, chemical composition of twenty-two common foods and 41
CANNON, M. D. See Sober, Maanering, Cannon, Elvehjem and Hart 503
Carbohydrate in diet, substitution of protein for, thiamine requirement of albino rat as influenced by ... 317
Carotene and vitamin A, absorption and retention of, by hens on normal and low fat rations 199
—— —— —— in rat, utilization of 57
Cataract and other ocular changes resulting from tryptophane deficiency 159
Cattle, young, with vitamin A deficiency, cystic pituitary in 15
CERECEDO, L. R., AND J. R. FOY. storage of pyridoxine in the rat 93
CHAMBERS, A. H. The respiratory quotient of the liver. I. Determination of the liver R.Q. in anesthetized dogs. II. Influence of certain diets on the R.Q. 331
Chemical composition of twenty-two common foods and comparison of analytical with calculated values of diets 41
Chick(s), liver concentrate as source of unrecognized vitamins required by 65
—— maintained on heated diet, factors required by 187
—— pyridoxine deficiency in 515
—— soybean protein as source of amino acids for 385
Chicken feathers, powdered, nutritional studies on 399
Children of early school age, study of ascorbic acid requirements of ... 25
—— pre-school, interrelationship of calcium, phosphorus and nitrogen in metabolism of 283
—— —— potassium, sodium and chlorine balances of, receiving medium and high protein diets ... 437
—— sulfur metabolism of 257
Chlorine, potassium and sodium balances of pre-school children receiving medium and high protein diets 437
Choline, lipotropic action of, relation of B-vitamins and dietary fat to 175
Citrus fruits, thiamine and riboflavin contents of 85
CLAUSEN, S. W., W. S. BAUM, A. B. MCCOORD, J. O. RYDEEN AND B. B. BREESE. The mobilization by alcohols of vitamin A from its stores in the tissues 1
Colostrum, milk and blood, ewes', ascorbic acid content of, and effect of ascorbic acid injections 121
Complex factors, B, inhibition of symbiotic synthesis of, by sulfonamides 427
—— vitamin B, effects of diet deficient in part of, upon men doing manual labor 585
Composition, chemical, of twenty-two common foods and comparison of analytical with calculated values of diets 41
Concentrate, liver, as a source of unrecognized vitamins required by chick 65
Content, ascorbic acid, of ewes' blood, colostrum and milk and effect of ascorbic acid injections 121
—— nicotinic acid, of meat 153
Contents, thiamine and riboflavin, of citrus fruits 85
CONVERSE, H. T. See Madsen, Hall and Converse 15
COX, G. See McKay, Patton, Ohlson, Pittman, Leverton, Marsh, Stearns and Cox 367
CRACAS, LOUIS J. See Light, Carcas, Olcott and Frey 427
Cystic pituitary in young cattle with vitamin A deficiency 15

DAFT, FLOYD S. See Lowry, Sebrell, Daft and Ashburn 73
DANN, W. J., AND P. HANDLER. The nicotinic acid content of meat ... 153
DARLING, R. C. See Johnson, Darling, Forbes, Brouha, Egaña and Graybiel 585
DAY, PAUL L. See Totter and Day ... 159
Deficiencies, thiamine, riboflavin, pyridoxine, and pantothenic acid, influence of, on nitrogen metabolism 405
Deficiency, pantothenic acid, effect of, on blood lipoids of dog 273
—— pyridoxine, in chicks 515
—— tryptophane, cataract and other ocular changes resulting from ... 159
—— vitamin A, cystic pituitary in young cattle with 15
—— —— B, effect of, on intestinal absorption of galactose in rat 495
Deficient rats, thiamine, polyneuropathy in, delayed by alcohol or whisky 73
Degeneration, sensory neuron, in pigs, IV. Protection afforded by pantothenic acid and pyridoxine 345
Dermatitis, rat, linoleic acid, pyridoxine and pantothenic acid in 225
Diet(s), basal, either low or high in phosphorus, salt mixture for use with 245
—— certain, influence of, on R.Q. (II). Respiratory quotient of liver. I. Determination of liver R.Q. 331
—— comparison of analytical with calculated values of, chemical composition of twenty-two common foods and 41
—— deficient in part of vitamin B complex, effects of, upon men doing manual labor 585
—— heated, factors required by chicks maintained on 187
—— of different composition, periodic administration of anterior pituitary extract as affecting metabolism of rats on 535
—— protein, medium and high, potassium, sodium and chlorine balances of pre-school children receiving .. 437

Diet(s), thiamine requirement of albino rat as influenced by substitution of protein for carbohydrate in ... 317
Dietary fat and B-vitamins, relation of, to lipotropic action of choline 175
—— phosphorus, absorption of, by rat, influence of aluminum sulfate and aluminum hydroxide upon 111
Dog(s) and rats, urinary excretion of riboflavin and nicotinic acid in, effect of level of protein intake upon 295
—— anesthetized, determination of liver R.Q. in. (I) and II. Influence of certain diets on R.Q. Respiratory quotient of liver ... 331
—— blood lipoids of, effect of pantothenic acid deficiency on 273
—— riboflavin requirement of 449
DYE, MARIE. See Hawks, Bray, Wilde and Dye 283
—— See Hawks, Bray, Hartt, Whittemore and Dye 437

EFFECT(S) of ascorbic acid injections, and ascorbic acid content of ewes' blood, colostrum and milk 121
—— of diet deficient in part of vitamin B complex upon men doing manual labor 585
—— of glucose and sucrose on respiratory quotient and muscular efficiency of exercise 307
—— of level of protein intake upon urinary excretion of riboflavin and nicotinic acid in dogs and rats 295
—— of pantothenic acid deficiency on blood lipoids of dog 273
—— of prolonged daily treatment of normal rats with saline anterior pituitary extract. I. Sexual differences in appetite, growth and organ weights 469
II. Protein and energy metabolism 481
—— of vitamin B deficiency on intestinal absorption of galactose in rat 495
Effectiveness of linoleic, arachidonic and linolenic acids in reproduction and lactation 213
Efficiency, muscular, and respiratory quotient of exercise, effect of glucose and sucrose on 307
EGAÑA, E. See Johnson, Darling, Forbes, Brouha, Egaña and Graybiel 585
ELVEHJEM, C. A. See Potter, Axelrod and Elvehjem 449
—— See Sober, Mannering, Cannon, Elvehjem and Hart 503
—— See Teply, Strong and Elvehjem 167
—— See Waisman, Mills and Elvehjem 187
Energy and protein metabolism. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. II. 481
ENGEL, R. W. The relation of B-vitamins and dietary fat to the lipotropic action of choline 175
Evaluation of nutritional status of a population group in Madrid, Spain, during summer of 1941 .. 557
Ewes blood, colostrum and milk, ascorbic acid content of, and effect of ascorbic acid injections 121
Excretion, urinary, or riboflavin and nicotinic acid in dogs and rats, effect of level of protein intake upon 295
Exercise, effect of glucose and sucrose on respiratory quotient and muscular efficiency of 307
Extract, anterior pituitary, periodic administration of, as affecting metabolism of rats on diets of different composition 535
—— saline anterior pituitary, effects of prolonged daily treatment of normal rats with. I. Sexual differences in appetite, growth and organ weights 469
II. Protein and energy metabolism 481

FACTORS, B complex, inhibition of symbiotic synthesis of, by sulfonamides 427
—— required by chicks maintained on a heated diet 187
Fat(s) body, in rat acrodynia 393
—— dietary, and B-vitamins, relation of, to lipotropic action of choline 175
—— low, and normal rations, absorption and retention of carotene and vitamin A by hens on 199
Feathers, powdered chicken, nutritional studies on 399
FIELD, HENRY, JR. See Melnick and Field 131
FOLLIS, RICHARD H., JR. See Wintrobe, Miller, Follis, Stein, Mushatt and Humphreys 345
Foods, common, twenty-two, chemical composition of, and comparison of analytical with calculated values of diets 41
—— nicotinic acid in 235
FORBES, W. H., See Johnson, Darling, Forbes, Brouha, Egaña and Graybiel 585
FORD, ZENAS W., JR. See Sure and Ford 405
FOSTER, CLAIRE. See Jones and Foster 245
FOSTER, JOHN E. See Satterfield, Bailey, Foster and Hostetler 121
FOX, minimum vitamin A requirement of 97
FOY, JOHN R. See Cerecedo and Foy 93
FRAPS, G. S. See Treichler, Kemmerer and Fraps 57
FREE, A. H., AND J. R. LEONARDS. The Effect of vitamin B deficiency on the intestinal absorption of galactose in the rat 495
FREY, CHARLES N. See Light, Cracas, Olcott and Frey 427
Fruits, citrus, thiamine and riboflavin contents of 85

GALACTOSE in rat, effect of vitamin B deficiency on intestinal absorption of 495
GALBRAITH, HARRY. See Hummel, Shepherd, Galbraith, Williams and Macy 41
Glucose and sucrose, effect of, on respiratory quotient and muscular efficiency of exercise 307
GRANDE (COVIAN), F. See Robinson, Janney and Grande (Covian) ... 557
GRAU, C. R. See Almquist, Mecchi, Kratzer and Grau 385
GRAYBIEL, A. See Johnson, Darling, Forbes, Brouha, Egaña and Graybiel 585
Growth, appetite and organ weights, sexual differences in. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. I. 469
—— rate and metabolism of rats 461
Guinea pig, nutrition of 503

HALL, SAMUEL R. See Madsen, Hall and Converse 15
HAMLIN, MARGARET T. See Scudi and Hamlin 273
HANDLER, PHILIP. See Dann and Handler 153
HARRIS, PHILIP L. See Remington, Harris and Smith 597
HART, E. B. See Sober, Mannering, Cannon, Elvehjem and Hart 503
HARTT, SYLVIA. See Hawks, Bray, Hartt, Whittemore and Dye ... 437
HAWKS, J. E., M. M. BRAY, M. O. WILDE AND M. DYE, WITH ASSISTANCE OF V. H. WILTGEN AND A. KILPATRICK. The interrelationship of calcium, phosphorus and nitrogen in the metabolism of preschool children 283
HAWKS, J. E., M. M. BRAY, S. M. HARTT, M. B. WHITTEMORE AND M. DYE. Potassium, sodium and chlorine balances of pre-school children receiving medium and high protein diets 437
Heat production and respiratory exchange, metabolism of arginine and leucine with special reference to 547
Heated diet, factors required by chicks maintained on 187
Hens on normal and low fat rations, absorption and retention of carotene and vitamin A by 199
HOFFMAN, OLIVE D. See Beach, Teague, Hoffman, Munks, Hummel, Williams and Macy 257
HOGAN, ALBERT G. See Richardson, Hogan and Karrasch 65
HOSTETLER, EARLE H. See Satterfield, Bailey, Foster and Hostetler 121
HUMMEL, F. C., M. L. SHEPHERD, H. GALBRAITH, H. H. WILLIAMS AND I. G. MACY. Chemical composition of twenty-two common foods and comparison of analytical with calculated values of diets 41
HUMMEL, FRANCES C. See Beach, Teague, Hoffman, Munks, Hummel, Williams and Macy 257
HUMPHREYS, STEWART. See Wintrobe, Miller, Follis, Stein, Mushatt and Humphreys 345
Hydroxide, aluminum, and aluminum sulfate, influence of, upon absorption of dietary phosphorus by rat 111

INDEX of nutritional status, thiamine clearance as 131
Influence of aluminum sulfate and aluminum hydroxide upon absorption of dietary phosphorus by rat 111
—— of thiamine, riboflavin, pyridoxine, and pantothenic acid deficiencies on nitrogen metabolism 405
Inhibition of symbiotic synthesis of B complex factors by sulfonamides .. 427
Injections, ascorbic acid, effect of, and ascorbic acid content of ewes' blood, colostrum and milk 121
Interrelationship of calcium, phosphorus and nitrogen in metabolism of pre-school children 283
Intestinal absorption of galactose in rat, effect of vitamin B deficiency on .. 495
—— synthesis of biotin in rat. Response of bacteria, yeast and rats to peroxide-treated biotin ... 523
Iodine metabolism and vitamin A in rat, relationship between 597

JANNEY, JOHN H. See Robinson, Janney and Grande (Covian) ... 557
JOHNSON, R. E., R. C. DARLING, W. H. FORBES, L. BROUHA, E. EGAÑA AND A. GRAYBIEL. The effects of a diet deficient in part of the vitamin B complex upon men doing manual labor 585
JONES, J. H., AND C. FOSTER. A salt mixture for use with basal diets either low or high in phosphorus 245

KARRASCH, RICHARD J. See Richardson, Hogan and Karrasch 65
KEMMERER, A. R. See Treichler, Kemmerer and Fraps 57
KIBLER, H. H., AND S. BRODY. Metabolism and growth rate of rats 461
KILPATRICK, ANNE. See Hawks, Bray, Wilde and Dye 283
KLEIN, JAMES R. See Sarett, Klein and Perlzweig 295
KRATZER, F. H. See Almquist, Mecchi, Kratzer and Grau 385
—— See Lepkovsky and Kratzer 515
KRISS, M., AND R. S. BOWMAN. The metabolism of arginine and leucine with special reference to respiratory exchange and heat production 547
KRISS, MAX. See Archer, Kriss and Voris 535
—— See Voris, Kriss, Marcy and Bowman 469, 481
KUMMEROW, FRED A. See Quackenbush, Kummerow and Steenbock 213
—— See Quackenbush, Steenbock, Kummerow and Platz 225

LACTATION and reproduction, effectiveness of linoleic, arachidonic, and linolenic acids in 213
LEONARDS, JACK R. See Free and Leonards 495
LEPKOVSKY, S., AND F. H. KRATZER. Pyridoxine deficiency in chicks ... 515
Leucine and arginine, metabolism of, with special reference to respiratory exchange and heat production 547
Level of protein intake, effect of, upon urinary excretion of riboflavin and nicotinic acid in dogs and rats 295
LEVERTON, R. M. See McKay, Patton Ohlson, Pittman, Leverton, Marsh, Stearns and Cox 367
LIGHT, R. F., L. J. CRACAS, C. T. OLCOTT AND C. N. FREY. Inhibition of the symbiotic synthesis of B complex factors by sulfonamides 427
Linoleic acid, pyridoxine and pantothenic acid in rat dermatitis 225
—— arachidonic, and linolenic acids, effectiveness of, in reproduction and lactation 213
Lipoids, blood, of dog, effect of pantothenic acid deficiency on 273
Lipotropic action of choline, relation of B-vitamins and dietary fat to 175
Liver concentrate as a source of unrecognized vitamins required by chick 65
—— respiratory quotient of. I. Determination of liver R.Q. in anesthetized dogs. II. Influence of certain diets on R.Q. 331
LOWRY, J. V., W. H. SEBRELL, F. S. DAFT AND L. L. ASHBURN. Polyneuropathy in thiamine deficient rats delayed by alcohol or whisky 73

McCOORD, A. B. See Clausen, Baum, McCoord, Rydeen and Breese 1
McKAY, H., M. B. PATTON, M. A. OHLSON, M. S. PITTMAN, R. M. LEVERTON, A. G. MARSH, G. STEARNS AND G. M. COX. Calcium, phosphorus and nitrogen metabolism of young college women ... 367
McVICAR, R. W., AND G. H. BERRYMAN. Nicotinic acid in foods ... 235
MACY, ICIE G. See Beach, Teague, Hoffman, Munks, Hummel, Williams and Macy 257
—— See Hummel, Shepherd, Galbraith, Williams and Macy 41
Madrid, Spain, evaluation of nutritional status of a population group in, during summer of 1941 557
MADSEN, L. L., S. R. HALL AND H. T. CONVERSE. Cystic pituitary in young cattle with vitamin A deficiency 15
Man, vitámin B_1 (thiamine) requirement of 139
MANNERING, G. J. See Sober, Mannering, Cannon, Elvehjem and Hart 503
Manual labor, effects of diet deficient in part of vitamin B complex upon men doing 585
MARCY, LAWSON F. See Voris, Kriss, Marcy and Bowman 469, 481
MARSH, A. G. See McKay, Patton, Ohlson, Pittman, Leverton, Marsh, Stearns and Cox 367
Meat, nicotinic acid content of 153
MECCHI, E. See Almquist, Mecchi, Kratzer and Grau 385
MELNICK, D. Vitamin B_1 (thiamine) requirement of man 139
MELNICK, D., AND H. FIELD, JR. Thiamine clearance as an index of nutritional status 131
Men doing manual labor, effects of diet deficient in part of vitamin B complex upon 585
Metabolism and growth rate of rats ... 461
—— calcium, phosphorus and nitrogen, of young college women 367
—— iodine, and vitamin A in rat, relationship between 597
—— nitrogen, influence of thiamine, riboflavin, pyridoxine and pantothenic acid deficiencies on 405
—— of arginine and leucine with special reference to respiratory exchange and heat production 547
—— of pre-school children, interrelationship of calcium, phosphorus and nitrogen in 283
—— of rats on diets of different composition, periodic administration of anterior pituitary extract as affecting 535
—— protein and energy. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. II. 481
—— sulfur, of children 257
Milk, blood and colostrum, ewes', ascorbic acid content of, and effect of ascorbic acid injections .. 121
MILLER, MITCHELL H. See Wintrobe, Miller, Follis, Stein, Mushatt and Humphreys 345
MILLS, RUSSELL C. See Waisman, Mills and Elvehjem 187
Minimum vitamin A requirement of fox 97
Mobilization by alcohols of vitamin A from its stores in the tissues ... 1
MUNKS, BERTHA R. See Beach, Teague, Hoffman, Munks, Hummel, Williams and Macy 257
Muscular efficiency and respiratory quotient of exercise, effect of glucose and sucrose on 307
MUSHATT, CECIL. See Wintrobe, Miller, Follis, Stein, Mushatt and Humphreys 345

NEURON, sensory, degeneration in pigs. IV. Protection afforded by pantothenic acid and pyridoxine .. 345
Nicotinic acid and riboflavin, urinary excretion of, in dogs and rats, effect of level of protein intake upon 295
—— —— content of meat 153
—— —— in foods 235
—— —— pantothenic acid and pyridoxine in wheat and wheat products 167
NIELSEN, E., G. M. SHULL AND W. H. PETERSON. Response of bacteria, yeast and rats to peroxide-treated biotin. Intestinal synthesis of biotin in the rat 523
Nitrogen, calcium and phosphorus, interrelationship of, in metabolism of pre-school children 283
—— —— —— —— metabolism of young college women 367
—— metabolism, influence of thiamine, riboflavin, pyridoxine, and pantothenic acid deficiencies on 405
Nutrition of guinea pig 503
Nutritional status of a population group in Madrid, Spain, during summer of 1941, evaluation of .. 557
—— —— thiamine clearance as an index of 131
—— studies on powdered chicken feathers 399

OCULAR changes, other, and cataract, resulting from tryptophane deficiency 159
OHLSON, M. A. See McKay, Patton, Ohlson, Pittman, Leverton, Marsh, Stearns and Cox 367
OLCOTT, CHARLES T. See Light, Cracas, Olcott and Frey 427
Organ weights, appetite and growth, sexual differences in. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. I. 469

PANTOTHENIC acid and pyridozine, protection afforded by, Sensory neuron degeneration in pigs. IV. 345
—— —— deficiency, effect of, on blood lipoids of dog 273
—— —— linoleic acid and pyridoxine in rat dermatitis 225
—— —— nicotinic acid and pyridoxine in wheat and wheat products 167
—— —— thiamine, riboflavin, and pyridoxine deficiencies, influence of, on nitrogen metabolism 405
PATTON, M. B. See McKay, Patton, Ohlson, Pittman, Leverton, Marsh, Stearns and Cox 367
Periodic administration of anterior pituitary extract as affecting metabolism of rats on diets of different composition 535
PERLZWEIG, WILLIAM A. See Sarett, Klein and Perlzweig 295

Peroxide-treated biotin, response of bacteria, yeast and rats to. Intestinal synthesis of biotin in rat 523
PETERSON, W. H. See Nielsen, Shull and Peterson 523
Phosphorus, basal diets either low or high in, a salt mixture for use with 245
—— calcium and nitrogen, interrelationship of, in metabolism of preschool children 283
—— —— —— —— metabolism of young college women 367
—— dietary, absorption of, by rat, influence of aluminum sulfate and aluminum hydroxide upon ... 111
Pig, guinea, nutrition of 503
Pigs, sensory neuron degeneration in. IV. Protection afforded by pantothenic acid and pyridoxine 345
PITTMAN, M. S. See McKay, Patton, Ohlson, Pittman, Leverton, Marsh, Stearns and Cox 367
Pituitary, cystic, in young cattle with vitamin A deficiency 15
—— extract, anterior, periodic administration of, as affecting metabolism of rats on diets of different composition 535
—— —— saline anterior, effects of prolonged daily treatment of normal rats with. I. Sexual differences in appetite, growth and organ weights 469
II. Protein and energy metabolism 481
PLATZ, BLANCHE R. See Quackenbush, Steenbock, Kummerow and Platz 225
POLSKIN, LOUIS J. See Russell, Taylor, Walker and Polskin 199
Polyneuropathy in thiamine deficient rats delayed by alcohol or whisky 73
Potassium, sodium and chlorine balances of pre-school children receiving medium and high protein diets ... 437
POTTER, R. L., A. E. AXELROD AND C. A. ELVEHJEM. The riboflavin requirement of the dog 449
Powdered chicken feathers, nutritional studies on 399
Protein and energy metabolism. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. II. 481
—— diets, medium and high, potassium, sodium and chlorine balances of pre-school children receiving .. 437
—— intake, effect of level of, upon urinary excretion of riboflavin and nicotinic acid in dogs and rats 295
—— soybean, as source of amino acids for chick 385
—— substitution of, for carbohydrate in diet, thiamine requirement of albino rat as influenced by 317
Pyridoxine and pantothenic acid, protection afforded by. Sensory neuron degeneration in pigs. IV. 345
—— deficiency in chicks 515
—— in rat, storage of 93
—— linoleic acid and pantothenic acid in rat dermatitis 225
—— nicotinic acid and pantothenic acid in wheat and wheat products 167
—— thiamine, riboflavin and pantothenic acid deficiencies, influence of, on nitrogen metabolism 405

QUACKENBUSH, F. W., F. A. KUMMEROW AND H. STEENBOCK. The effectiveness of linoleic, arachidonic, and linolenic acids in reproduction and lactation 213
QUACKENBUSH, F. W., H. STEENBOCK, F. A. KUMMEROW AND B. R. PLATZ. Linoleic acid, pyridoxine and pantothenic acid in rat dermatitis 225
QUACKENBUSH, F. W., AND H. STEENBOCK. Body fats in rat acrodynia 393
Quotient, respiratory, and muscular efficiency of exercise, effect of glucose and sucrose on 307
—— —— of liver. I. Determination of liver R.Q. in anesthetized dogs. II. Influence of certain diets in R.Q. 331

RAT(S), absorption of dietary phosphorus by, influence of aluminum sulfate and aluminum hydroxide upon 111
—— acroydnia, body fats in 393
—— albino, thiamine requirement of, as influenced by substitution of protein for carbohydrate in diet 317
—— and dogs, urinary excretion of riboflavin and nicotinic acid in, effect of level of protein intake upon 295
—— dermatitis, linoleic acid, pyridoxine and pantothenic acid in 225
—— effect of vitamin B deficiency on intestinal absorption of galactose in 495
—— intestinal synthesis of biotin in. Response of bacteria, yeast and rats to peroxide-treated biotin ... 523
—— metabolism and growth rate of .. 461
—— —— of, on diets of different composition, periodic administration of anterior pituitary extract as affecting 535
—— normal, effects of prolonged daily treatment of, with saline anterior pituitary extract. I. Sexual differences in appetite, growth and organ weights 469
II. Protein and energy metabolism 481
—— relationship between vitamin A and iodine metabolism in 597
—— storage of pyridoxine in 93
—— thiamine deficient, polyneuropathy in, delayed by alcohol or whisky 73
—— utilization of carotene and vitamin A in 57
Rate, growth, and metabolism of rats 461
Rations, normal and low fat, absorption and retention of carotene and vitamin A by hens on 199
Relation of B-vitamins and dietary fat to lipotropic action of choline ... 175
Relationship between vitamin A and iodine metabolism in rat 597
REMINGTON, R. E., P. L. HARRIS AND C. L. SMITH. Relationship between vitamin A and iodine metabolism in the rat 597
Reproduction and lactation, effectiveness of linoleic, arachidonic, and linolenic acids in 213
Requirement(s), ascorbic acid, of children of early school age, study of 25
—— minimum vitamin A, of fox ... 97
—— riboflavin, of dog 449
—— thiamine, of albino rat as influenced by substitution of protein for carbohydrate in diet 317
—— vitamin B_1 (thiamine), of man .. 139
Respiratory exchange and heat production, metabolism of arginine and leucine with special reference to 547

Respiratory quotient and muscular efficiency of exercise, effect of glucose and sucrose on 307
—— —— of liver. I. Determination of liver R.Q. in anesthetized dogs. II. Influence of certain diets on R.Q. 331
Retention and absorption of carotene and vitamin A by hens on normal and low fat rations 199
Riboflavin and nicotinic acid, urinary excretion of, in dogs and rats, effect of level of protein intake upon 295
—— and thiamine contents of citrus fruits 85
—— requirement of dog 449
—— thiamine, pyridoxine, and pantothenic acid deficiencies, influence of, on nitrogen metabolism 405
RICHARDSON, L. R., A. G. HOGAN AND R. J. KARRASCH. A liver concentrate as a source of unrecognized vitamins required by the chick 65
ROBERTS, LYDIA J. See Roberts and Roberts 25
ROBERTS, V. M., AND L. J. ROBERTS. A study of the ascorbic acid requirements of children of early school age 25
ROBINSON, W. D., J. H. JANNEY AND F. GRANDE (COVIAN). An evaluation of the nutritional status of a population group in Madrid, Spain, during the summer of 1941 557
ROUTH, J. I. Nutritional studies on powdered chicken feathers 399
RUSSELL, W. C., M. W. TAYLOR, H. A. WALKER AND L. J. POLSKIN. The absorption and retention of carotene and vitamin A by hens on normal and low fat rations 199
RYDEEN, J. O. See Clausen, Baum, McCoord, Rydeen and Breese ... 1

SALINE anterior pituitary extract, effects of prolonged daily treatment of normal rats with. I. Sexual differences in appetite, growth and organ weights 469
II. Protein and energy metabolism 481
Salt mixture for use with basal diets either low or high in phosphorus 245
SARETT, H. P., J. R. KLEIN AND W. A. PERLZWEIG. The effect of the level of protein intake upon the urinary excretion of riboflavin and nicotinic acid in dogs and rats .. 295
SATTERFIELD, G. H., E. A. BAILEY, JR., J. E. FOSTER AND E. H. HOSTETLER. The ascorbic acid content of ewes' blood, colostrum and milk and the effect of ascorbic acid injections 121
SCUDI, J. V., AND M. T. HAMLIN. The effect of pantothenic acid deficiency on the blood lipoids of the dog ... 273
SEBRELL, WILLIAM H. See Lowry, Sebrell, Daft and Ashburn 73
Sensory neuron degeneration in pigs. IV. Protection afforded by pantothenic acid and pyridoxine 345
Sexual differences in appetite, growth and organ weights. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. I. 469
SHEPHERD, MARION L. See Hummel, Shepherd, Galbraith, Williams and Macy 41
SHULL, G. M. See Nielsen, Shull and Peterson 523
SMITH, CECIL L. See Remington, Harris and Smith 597
SMITH, S. E. The minimum vitamin A requirement of the fox 97
SOBER, H. A., G. J. MANNERING, M. D. CANNON, C. A. ELVEHJEM AND E. B. HART. Nutrition of the guinea pig 503
Sodium, potassium and chlorine balances of pre-school children receiving medium and high protein diets 437
Soybean protein as source of amino acids for chick 385
Spain (Madrid), evaluation of nutritional status of a population group in, during summer of 1941 557
STEARNS, G. See McKay, Patton, Ohlson, Pittman, Leverton, Marsh, Stearns and Cox 367
STEENBOCK, HARRY. See Quackenbush, Kummerow and Steenbock 213
—— See Quackenbush, Steenbock, Kummerow and Platz 225
—— See Quackenbush and Steenbock 393
STEIN, HAROLD J. See Wintrobe, Miller, Follis, Stein, Mushatt and Humphreys 345
Storage of pyridoxine in the rat 93
STREET, H. R. The influence of aluminum sulfate and aluminum hydroxide upon the absorption of dietary phosphorus by the rat .. 111
STRONG, FRANK M. See Teply, Strong and Elvehjem 167
Study of the ascorbic acid requirements of children of early school age ... 25
Sucrose and glucose, effect of, on respiratory quotient and muscular efficiency of exercise 307
Sulfate, aluminum, and aluminum hydroxide, influence of, upon absorption of dietary phosphorus by rat 111
Sulfonamides, inhibition of symbiotic synthesis of B complex factors by 427
Sulfur metabolism of children 257
SURE, B., AND Z. W. FORD, JR. The influence of thiamine, riboflavin, pyridoxine, and pantothenic acid deficiencies on nitrogen metabolism 405
Synthesis, intestinal, of biotin in rat. Response of bacteria, yeast and rats to peroxide-treated biotin ... 523
—— symbiotic, of B complex factors by sulfonamides, inhibition of 427

TAYLOR, MILTON W. See Russell, Taylor, Walker and Polskin 199
TEAGUE, DWIGHT M. See Beach, Teague, Hoffman, Munks, Hummel, Williams and Macy 257
TEPLY, L. J., F. M. STRONG AND C. A. ELVEHJEM. Nicotinic acid, pantothenic acid and pyridoxine in wheat and wheat products 167
Thiamine and riboflavin contents of citrus fruits 85
—— clearance as an index of nutritional status 131
—— deficient rats, polyneuropathy in, delayed by alcohol or whisky ... 73
—— requirement of albino rat as influenced by substitution of protein for carbohydrate in diet 317
—— riboflavin, pyridoxine, and pantothenic acid deficiencies, influence of, on nitrogen metabolism 405
—— (vitamin B_1) requirement of man 139
THOMAS, ARTHUR W. See Bailey and Thomas 85

Tissues, mobilization by alcohols of vitamin A from its stores in ... 1
TOTTER, J. R., AND P. L. DAY. Cataract and other ocular changes resulting from tryptophane deficiency 159
TREICHLER, R., A. R. KEMMERER AND G. S. FRAPS. The utilization of carotene and vitamin A in the rat 57
Tryptophane deficiency, cataract and other ocular changes resulting from 159

URINARY excretion of riboflavin and nicotinic acid in dogs and rats, effect of level of protein intake upon ... 295
Utilization of carotene and vitamin A in the rat ... 57

VALUES of diets, comparison of analytical with calculated, chemical composition of twenty-two common foods and ... 41
Vitamin(s) A and carotene, absorption and retention of, by hens on normal and low fat rations ... 199
—— A and carotene in rat, utilization of ... 57
—— A and iodine metabolism in rat, relationship between ... 597
—— A deficiency, cystic pituitary in young cattle with ... 15
—— A, minimum, requirement of fox 97
—— A, mobilization by alcohols of, from its stores in tissues ... 1
—— B, and dietary fat, relation of, to lipotropic action of choline ... 175
—— B complex, effects of diet deficient in part of, upon men doing manual labor ... 585
—— B deficiency, effect of, on intestinal absorption of galactose in rat ... 495
—— B_1 (thiamine) requirement of man 139
—— unrecognized, required by chick, liver concentrate as source of ... 65
VORIS, L., M. KRISS, L. F. MARCY AND R. S. BOWMAN. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. I. Sexual differences in appetite, growth and organ weights 469
II. Protein and energy metabolism 481
VORIS, LEROY. See Archer, Kriss and Voris ... 535

WAINIO, W. W. The thiamine requirement of the albino rat as influenced by the substitution of protein for carbohydrate in the diet ... 317
WAISMAN, H. A., R. C. MILLS AND C. A. ELVEHJEM. Factors required by chicks maintained on a heated diet ... 187
WALKER, HARRY A. See Russell, Taylor, Walker and Polskin ... 199
Weights, organ, appetite and growth, sexual differences in. Effects of prolonged daily treatment of normal rats with saline anterior pituitary extract. I. ... 469
Wheat and wheat products, nicotinic acid, pantothenic acid and pyridoxine in ... 167
Whisky or alcohol, polyneuropathy in thiamine deficient rats delayed by 73
WHITTEMORE, MARGARET B. See Hawks, Bray, Hartt, Whittemore and Dye ... 437
WILDE, MARJORIE O. See Hawks, Bray, Wilde and Dye ... 283
WILLIAMS, HAROLD H. See Beach, Teague, Hoffman, Munks, Hummel, Williams and Macy ... 257
—— See Hummel, Shepherd, Galbraith, Williams and Macy ... 41
WILTGEN, VEDA H. See Hawks, Bray, Wilde and Dye ... 283
WINTROBE, M. M., M. H. MILLER, R. H. FOLLIS, JR., H. J. STEIN, C. MUSHATT AND S. HUMPHREYS. Sensory neuron degeneration in pigs. IV. Protection afforded by calcium pantothenate and pyridoxine ... 345
Women, young college, calcium, phosphorus and nitrogen metabolism of ... 367
WRIGHTINGTON, M. The effect of glucose and sucrose on the respiratory quotient and muscular efficiency of exercise ... 307

YEAST, bacteria and rats, response of, to peroxide-treated biotin. Intestinal synthesis of biotin in rat 523

The soldier who left 960 dependents

WHEN THIS physician entered the army, he left behind some 960 people who formerly depended on him for medical care.

This has thrown a heavy extra burden on the doctors left behind to keep the home front strong and healthy. They have accepted the resulting extra hours and overwork without complaining. But there is a limit to human endurance and there are but 24 hours in every day.

This is one of the chief reasons why we feel that the National Nutrition Program is so important. For its purpose, as you know, is to make Americans healthier through better nutrition. And the healthier people are, the less will be the demands on the crowded time of our doctors.

The importance of milk and milk products among foods recommended by the National Nutrition Program, enables a company like Borden to be of real service.

Our research for years has centered on milk and its place in human nutrition. Men in our laboratories have already made many contributions in the field of milk research and we have every reason to expect them to make more.

Their work has had its practical side, too. Perhaps their outstanding accomplishment along this line has been the development and perfection of Borden's unique Quality Control.

Quality Control is our name for the rigid system of laboratory checks and balances which safeguards the purity, uniformity, and quality of every Borden product in every phase of production.

Biolac or Beta Lactose, Evaporated Milk or Ice Cream, Quality Control makes the name Borden a mark of reassurance on any milk product.

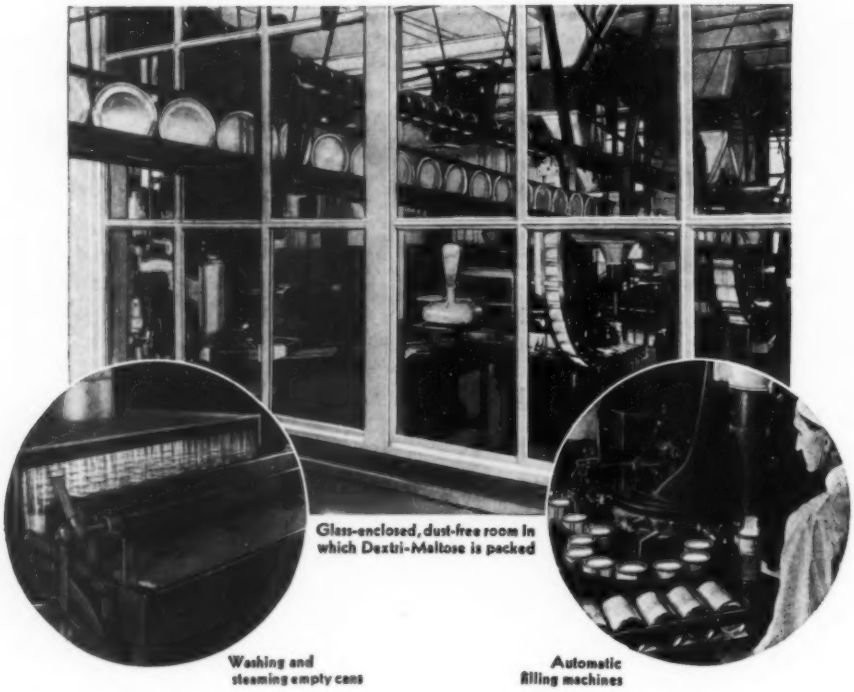
THE BORDEN COMPANY

350 MADISON AVENUE • NEW YORK CITY

• • •

BORDEN . . . *better nutrition through milk research*

Extreme Cleanliness Used in Packing DEXTRI-MALTOSE



Glass-enclosed, dust-free room in which Dextri-Maltose is packed

Washing and steaming empty cans

Automatic filling machines

THE EXACTING CARE used in manufacturing Dextri-Maltose is maintained, even to the final packing and wrapping. The empty cans are washed inside and outside by hot water under pressure, then steamed and dried. Cans are filled in a glass-enclosed, air-conditioned room, the atmosphere of which is washed to free it from dust and bacteria. Enclosed conveyors leading to this room send Dextri-Maltose to machines which automatically fill and close the cans without human handling of the product. The cans are again washed and inspected for imperfections. At this step specimen cans are selected for bacteriological examination to insure that there has been no break in the sanitary control. Finally the cans are wrapped to prevent the covers from accumulating dust. Dextri-Maltose is then held in storage and released for the market only after final approval from the bacteriological laboratory. At every stage in the manufacture of Dextri-Maltose our effort is concentrated on supplying the physician with the carbohydrate that will best promote and maintain the health of his patients.

"The Measure of Economy is Value, Not Price"

MEAD JOHNSON & COMPANY, Evansville, Indiana, U. S. A.

Please enclose professional card when requesting samples of Mead Johnson products to cooperate in preventing their reaching unauthorized persons

To you who are taking an active part in promoting the national nutrition program . . .

All Kellogg Cereals meet the requirements of the U.S. Official Nutrition Food Rules

★ Cereals . . . the right *kind* of cereals . . . have an important place in America's wartime nutrition program, according to the Nutrition Division, Office of Defense Health and Welfare Services.

The *type* of cereals specified by the Official Nutrition Food Rules are those which are *whole grain* products, or which have been restored to original *whole grain nutritive values.*

Every cereal in the famous Kellogg Line meets one of these two requirements . . . is made from whole grain, or is restored to whole grain values in thiamin, niacin and iron.

This fact should be of special significance to you. Kellogg's Cereals have long been the country's most popular favorites. They are already a breakfast *habit* with millions of American families . . . a habit that *need not be changed* for full co-operation with the nutrition program.

Moreover, Kellogg's Cereals also encourage greater consumption of milk and fruit; help save sugar, because they are pre-sweetened; help save time and fuel, because they require no cooking.

KELLOGG'S CEREALS

Made in Battle Creek, Michigan

Vitamins for Laboratory Use

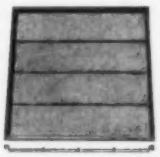
l-Ascorbic Acid	2 grams, 10 grams
Calcium d-Pantothenate	1 gram, 5 grams
Choline Chloride	10 grams, 100 grams
2-Methyl-1, 4-Naphthoquinone	1 gram
Nicotinic Acid	5 grams, 50 grams
Nicotinic Acid Amide	4 grams, 10 grams
Pyridoxine Hydrochloride	1 gram
d—Riboflavin	1 gram
Thiamine Hydrochloride	5 grams

Price List on request to:

LEDERLE LABORATORIES, INC.

30 Rockefeller Plaza, New York, N. Y.

THE WISTAR INSTITUTE SLIDE TRAY



Section through two trays, showing nesting feature

Ideal tray for displaying or storing slides. Carries 48–1″, 32–1½″, or 24–2″ slides. Visible at a glance. Nesting feature permits trays to be stacked. Dust proof. Entirely of metal, unbreakable and easily kept clean. Form compact storage units.

Price $1.00 each

Send orders to

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
Woodland Avenue and Thirty-sixth Street
Philadelphia, Pa.

LABCO RELIABILITY

ESTABLISHES CONFIDENCE

Labco Rice Polish derivatives carrying concentrated natural vitamin factors at standardized and uniform levels have assumed an indispensable status as ration adjuncts and supplements in numerous biological research and control laboratories.

Prices and Information on Request

THE BORDEN COMPANY
LABCO PRODUCTS DEPARTMENT
350 Madison Avenue, New York, N. Y.

NOTICE TO CONTRIBUTORS

THE JOURNAL OF NUTRITION appears monthly for the publication of original research bearing on the subject of nutrition and occasional reviews of the literature dealing with this subject.

THE JOURNAL OF NUTRITION is the official organ of the American Institute of Nutrition organized for the dissemination of scientific knowledge regarding nutrition. The officers of the Institute are, L. A. Maynard, President; Howard B. Lewis, Vice-President; Arthur H. Smith, Secretary; W. H. Sebrell, Treasurer.

Preliminary notices will not be accepted. No paper which has already appeared will be accepted nor will simultaneous publication elsewhere be permitted.

The paper must be accompanied by an author's abstract not to exceed 225 words in length, which will appear on the advance abstract cards of the Bibliographic Service of The Wistar Institute in advance of complete publication. Nothing can be done with the manuscript until the abstract is received.

Manuscripts and drawings should be sent by express prepaid or by registered mail to the Editor, DR. GEORGE R. COWGILL, Yale University School of Medicine, 333 Cedar Street, New Haven, Conn.

Manuscripts should be typewritten in double spacing on one side of paper $8\frac{1}{2} \times 11$ inches, and should be packed flat — not rolled or folded. The original, not carbon, copy should be sent. The original drawings, not photographs of drawings, should accompany the manuscript. When photographs are used for halftone reproduction glossy prints should be sent. Authors should indicate on the manuscript the approximate position of text figures.

Manuscripts and drawings should be submitted in complete and finished form with the author's complete address. All drawings should be marked with the author's name. The Wistar Institute reserves the privilege of returning to the author for revision approved manuscript and illustrations which are not in proper finished form for the printer; or, if the author so desires, The Wistar Institute will prepare the manuscript and charge the author the cost of this work. When the amount of tabular and illustrative material is judged to be excessive, or unusually expensive, authors may be requested to pay the excess cost.

The tables, quotations (extracts of over five lines), and all other subsidiary matter usually set in type smaller than the text, should be typewritten on separate sheets and placed with the text in correct sequence. Footnotes should not be in with the text (reference numbers only), but typewritten continuously on separate sheets, and numbered consecutively. Explanations of figures should be treated in the same manner, and, like footnotes, should be put at the end of the text copy. A condensed title for running page headline, not to exceed thirty-five letters and spaces, should be given. Citations of literature should be made in the text by numerals indicating the year of publication. Authors' names (followed by year, title, etc.) should be arranged alphabetically in a list at the end of the paper only.

Figures should be drawn for reproduction as line or halftone engravings, unless the author is prepared to defray the additional cost of a more expensive form of illustration. In grouping the drawings it should be borne in mind that, after the reduction has been made, text figures are not to exceed the dimensions of the printed matter on the page, $4\frac{1}{2} \times 6\frac{1}{2}$ inches. Single plates may be $5 \times 7\frac{1}{2}$ inches, or less. No double plates will be accepted.

Figures should be numbered from 1 up, beginning with the text figures and continuing through the plates. The reduction desired should be clearly indicated on the margin of the drawing.

All drawings intended for photographic reproduction either as line engravings (black-ink pen lines and dots) or halftone plates (wash and brush work) should be made on white or blue-white paper or bristol board — not on cream-white or yellow-toned. Photographs intended for halftone reproduction should be securely mounted with colorless paste — never with glue, which discolors the photograph.

Galley proofs and engraver's proofs of figures are sent to the author. All corrections should be clearly marked thereon.

The journal furnishes the author fifty reprints, with covers, of the paper gratis. Additional copies may be obtained according to rates which will be sent the author as soon as the manuscript has been examined.

THIS NUMBER COMPLETES VOLUME 24

THE JOURNAL OF NUTRITION

VOL. 24 DECEMBER 10, 1942 No. 6

CONTENTS

H. A. SOBER, G. J. MANNERING, M. D. CANNON, C. A. ELVEHJEM AND E. B. HART. Nutrition of the guinea pig 503

SAMUEL LEPKOVSKY AND F. H. KRATZER. Pyridoxine deficiency in chicks. One figure 515

EDWARD NIELSEN, G. M. SHULL AND W. H. PETERSON. Response of bacteria, yeast and rats to peroxide-treated biotin. Intestinal synthesis of biotin in the rat. One figure 523

HELEN E. ARCHER, MAX KRISS AND LEROY VORIS. Periodic administration of anterior pituitary extract as affecting the metabolism of rats on diets of different composition 535

MAX KRISS AND ROBERT S. BOWMAN. The metabolism of arginine and leucine with special reference to respiratory exchange and heat production 547

WILLIAM D. ROBINSON, JOHN H. JANNEY AND FRANCISCO GRANDE (COVIAN). An evaluation of the nutritional status of a population group in Madrid, Spain, during the summer of 1941 557

R. E. JOHNSON, R. C. DARLING, W. H. FORBES, L. BROUHA, E. EGAÑA AND A. GRAYBIEL. The effects of a diet deficient in part of the vitamin B complex upon men doing manual labor. One figure 585

ROE E. REMINGTON, PHILIP L. HARRIS AND CECIL L. SMITH. Relationship between vitamin A and iodine metabolism in the rat 597

PRESS OF
THE WISTAR INSTITUTE
OF ANATOMY AND BIOLOGY
PHILADELPHIA

Printed in the United States of America